Europäisches Patentamt

(19) European Patent Office

Office européen des brevets

(11) **EP 1 193 270 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(54) **Pyrrolobenzodiazepines**

Pyrrolobenzodiazepine

Pyrrolobenzodiazépines

(72) Inventors:
• Thurston, David Edwin, School of Pharmacy
London WC1N 1AX (GB)
• Howard, Philip Wilson, School of Pharmacy
London WC1N 1AX (GB)

(56) References cited:
GB-A- 1 299 198

• CHEMICAL ABSTRACTS, vol. 90, no. 1, 1979
Columbus, Ohio, US; abstract no. 4427a, H.
UMEZAWA ET AL.: "Mazethramycins" page 428;
XP002135056 & JP 07 882792 A
(MICROBIOCHEMICAL RESEARCH
FOUNDATION)

• CHEMICAL ABSTRACTS, vol. 98, no. 9, 1983
Columbus, Ohio, US; abstract no. 72145x,
FUJISAWA: "Benzodiazepine derivatives" page
638; XP002135057 -& DATABASE CHEMABS
[Online] Chemical Abstracts Service, Columbus,
Ohio; FUSISAWA PHARMACEUTICAL CO.,
LTD.: "Benzodiazepine derivatives" Database
accession no. 98:72145 CA XP002189097 & JP
57 131791 A (FUJISAWA)

• CHEMICAL ABSTRACTS, vol. 126, no. 13, 1997
Columbus, Ohio, US; abstract no. 171573p, I.
O'NEIL ET AL: "The synthesi of functionalized
pyrrolo[2,1-][1,4]benzodiazepines" page 618;
XP002135261 -& DATABASE CHEMICAL
ABSTRACTS [Online] Chemical Abstracts
Service, Columbus; CA 126:171573,
XP002189098 & SYNLETT, no. 1, 1997, pages
75-78,

• CHEMICAL ABSTRACTS, vol. 114, no. 25, 1991
Columbus, Ohio, US; abstract no. 239940r, J..
FARMER ET AL.: "DNA binding properties of a
new class of linked anthramycin analogs" page
25; XP002135262 -& DATABASE CHEMABS
[Online] Chemical Abstracts Servive, Columbus;
FARMER J. ET AL: "DNA binding properties of a
new class of linked anthramacyn analogs"
Database accession no. 114:239940
XP002189099 & NUCLEIC ACIDS RES., vol. 19,
no. 4, 1991, pages 899-904,

• S.M. COURTNEY ET AL.: "A new convenient
procedure for the synthesis of
pyrrolo[2,1-c][1,4]benzodiazepines"
TETRAHEDRON LETTERS., vol. 34, no. 33, 1993,
pages 5327-28, XP002135259 ELSEVIER
SCIENCE PUBLISHERS, AMSTERDAM., NL
ISSN: 0040-4039

BEST AVAILABLE COPY

- S.J. GREGSON ET AL.: "Synthesis of a novel C2/C2'-exo unsaturated pyrrolobenzodiazepine cross-linking agent with remarkable DNA binding affinity and cytotoxicity" CHEMICAL COMMUNICATIONS., 1999, pages 797-798, XP002135055 ROYAL SOCIETY OF CHEMISTRY., GB ISSN: 1359-7345
- O'NEIL I A ET AL: "DPPE: A Convenient Replacement for Triphenylphosphine in the Staudinger and Mitsunobu Reactions" TETRAHEDRON LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 39, no. 42, 15 October 1998 (1998-10-15), pages 7787-7790, XP004134309 ISSN: 0040-4039
- GUIOTTO A ET AL: "Synthesis of novel C7-aryl substituted pyrrolo[2,1-c][1,4]benzodiazepines (PBDs) via Pro-N10-troc protection and suzuki coupling" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS,GB,OXFORD, vol. 8, no. 21, 3 November 1998 (1998-11-03), pages 3017-3018, XP004141866
- D.E. THURSTON ET AL.: "Effect of A-ring modifications on the DNA-binding behavior and citotoxicity of pyrrolo[2,1-c][1,4]benzodiazepines" JOURNAL OF MEDICINAL CHEMISTRY., vol. 42, 1999, pages 1951-1964, XP002135260 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US ISSN: 0022-2623

Description

Background to the invention

[0001] Some pyrrolobenzodiazepines (PBDs) have the ability to recognise and bond to specific sequences of DNA; the preferred sequence is PuGPu. The first PBD antitumour antibiotic, anthramycin, was discovered in 1965 (Leimgruber *et al.,* 1965 *J. Am. Chem. Soc.,* **87,** 5793-5795; Leimgruber *et al.,* 1965 *J. Am. Chem. Soc.,* **87,** 5791-5793). Since then, a number of naturally occurring PBDs have been reported, and over 10 synthetic routes have been developed to a variety of analogues (Thurston et al., 1994 *Chem. Rev.* **1994,** 433-465). Family members include abbeymycin (Hochlowski *et al.,* 1987 *J. Antibiotics,* **40,** 145-148), chicamycin (Konishi *et al.,* 1984 *J. Antibiotics,* **37,** 200-206), DC-81 (Japanese Patent 58-180 487; Thurston *et al.,* 1990, *Chem. Brit.,* **26,** 767-772; Bose *et al.,* 1992 *Tetrahedron,* **48,** 751-758), mazethramycin (Kuminoto *et al.,* 1980 *J. Antibiotics,* **33,** 665-667), neothramycins A and B (Takeuchi *et al.,* 1976 *J. Antibiotics,* **29,** 93-96), porothramycin (Tsunakawa et *al.,* 1988 *J. Antibiotics,* **41,** 1366-1373), prothracarcin (Shimizu *et al,* 1982 *J. Antibiotics,* **29,** 2492-2503; Langley and Thurston, 1987 *J. Org. Chem.,* **52,** 91-97), sibanomicin (DC-102) (Hara *et al.,* 1988 *J. Antibiotics,* **41,** 702-704; Itoh *et al.,* 1988 *J. Antibiotics,* **41,** 1281-1284), sibiromycin (Leber *et al.,* 1988 *J. Am. Chem. Soc.,* **110,** 2992-2993) and tomamycin (Arima *et al.,* 1972 *J. Antibiotics,* **25,** 437-444). PBDs are of the general structure:
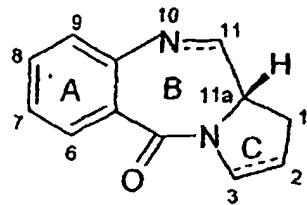
[0002] They differ in the number, type and position of substituents, in both their aromatic A rings and pyrrolo C rings, and in the degree of saturation of the C ring. In the B-ring there is either an imine (N=C), a carbinolamine(NH-CH(OH)), or a carbinolamine methyl ether (NH-CH(OMe)) at the N10-C11 position which is the electrophilic centre responsible for alkylating DNA. All of the known natural products have an (S)-configuration at the chiral C11a position which provides them with a right-handed twist when viewed from the C ring towards the A ring. This gives them the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, 1975 In *Antibiotics III.* Springer-Verlag, New York, pp. 3-11 ; Hurley and Needham-VanDevanter, 1986 *Acc. Chem. Res.,* **19,** 230-237). Their ability to form an adduct in the minor groove, enables them to interfere with DNA processing, hence their use as antitumour agents.

Disclosure of the invention

[0003] A first aspect of the present invention relates to a compound of formula:
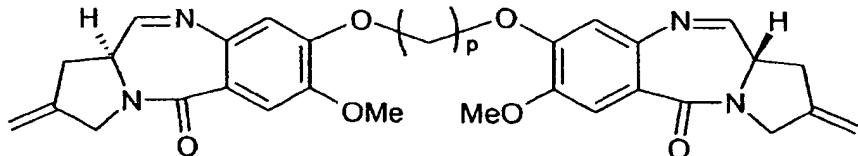
wherein p is 3.
[0004] This compound is 1,1'-[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11-tetrahydro-5*H*-pyrrolo[2,1-c][1,4]benzodiazepin-5-one].
[0005] A second aspect of the present invention is a compound as described in the first aspect of the invention for use in a method of treatment by therapy. Conditions which may be treated include gene-based diseases, including, for example, neoplastic diseases and Alzheimer's Disease, and also bacterial, parasitic and viral infections. Any condition which may be treated by the regulation of gene expression may be treated using compounds of the invention. In accordance with this aspect of the present invention, the compounds provided may be administered to individuals.

Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage, is within the responsibility of general practitioners and other medical doctors.

[0006] A compound may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

[0007] Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may comprise, in addition to the active ingredient, i.e. a compound of the first aspect a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous, or intravenous.

[0008] Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may comprise a solid carrier or an adjuvant. Liquid pharmaceutical compositions generally comprise a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included. A capsule may comprise a solid carrier such a gelatin.

[0009] For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

[0010] A third aspect of the present invention is a pharmaceutical composition containing a compound of the first aspect and a pharmaceutically acceptable carrier or diluent. The preparation of pharmaceutical compositions is described in relation to the second aspect of the invention above.

[0011] A fourth aspect of the present invention provides the use of a compound of the first aspect to prepare a medicament for the treatment of a gene-based disease, preferably a proliferative disease.. The compound of formula may be provided together with a pharmaceutically acceptable carrier or diluent. The compounds may be used for the selective killing of oxic and hypoxic tumour cells in methods for the treatment of cancers, for example leukemias and particularly solid cancers including colon, CNS, renal, and lung tumours, including small cell lung carcinoma, and melanomas. In particular, compounds of the first aspect may be used for the selective killing of lung, colon, and CNS tumours and melanomas.

[0012] A further aspect of the present invention provides the use of a compound of the first aspect to prepare a medicament for the treatment of a viral, parasitic or bacterial infection. The preparation of a medicament is described in relation to the second aspect of the invention above.

[0013] In further aspects, the invention provides processes for preparing compounds according to the first aspect of the present invention.

[0014] Aspects of the invention will now be further described with reference to the accompanying drawings in which:

Figures 1 to 3a/b are synthesis routes for compounds of the present invention.

Preferred General Synthetic Strategies

[0015] A key step in a preferred route to a compound of the present invention is a cyclisation to produce the B-ring, involving generation of an aldehyde (or functional equivalent thereof) at what will be the 11-position, and attack thereon by the Pro-N10-nitrogen:
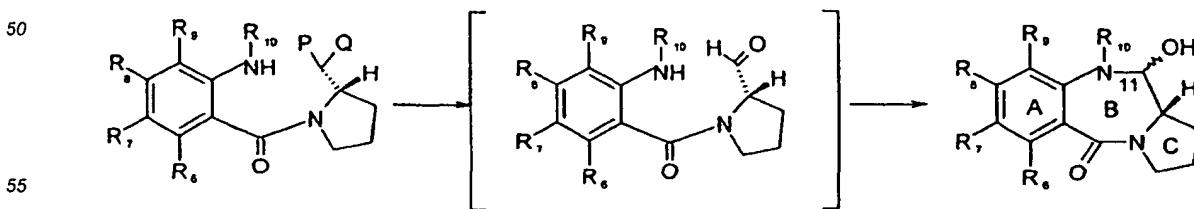
[0016] In this structure, no C-ring substitution is shown. R_8 represents the dimer bridge. R_{10} is a nitrogen protecting

group, preferably with a carbamate functionality bonded to the nitrogen of the PBD. The "masked aldehyde" -CPQ may be an acetal or thioacetal (possibly cyclic), in which case the cyclisation involves unmasking. Alternatively, the masked aldehyde may be an aldehyde precursor, such as alcohol -CHOH, in which case the reaction involves oxidation, e.g. by means of TPAP or DMSO (Swern oxidation).

[0017] The masked aldehyde compound can be produced by condensing a corresponding 2-substituted pyrrolidine with a 2-nitrobenzoic acid:
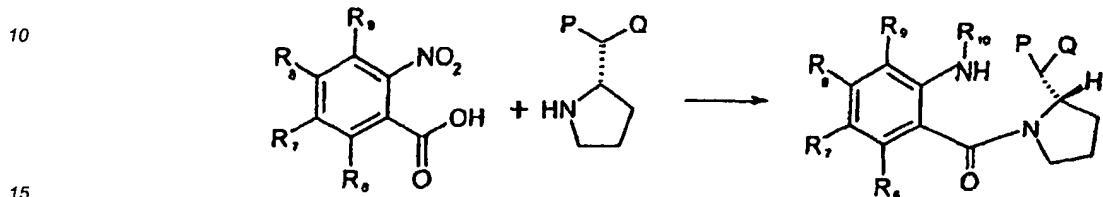
[0018] The nitro group can then be reduced to -NH₂ and protected by reaction with a suitable reagent, e.g. a chloroformate, which provides the removable nitrogen protecting group in the synthesis route.

[0019] A process involving the oxidation-cyclization procedure is illustrated in scheme 1 with respect to monomers which are outside the present invention(an alternative type of cyclisation will be described later with reference to scheme 2).
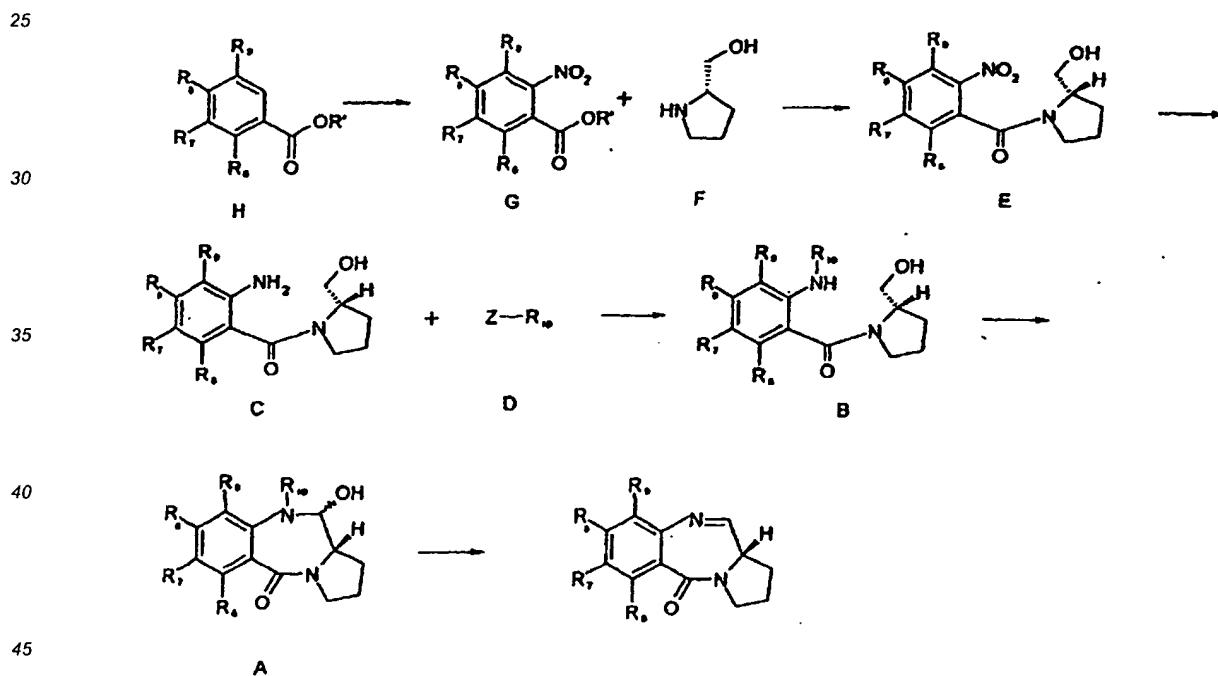
Scheme 1

[0020] The imine/carbinolamine bond in the PBD **(A)** can be unprotected by standard methods to yield the desired compound, e.g. if R₁₀ is Alloc, then the deprotection is carried out using palladium to remove the N10 protecting group, followed by the elimination of water to give the imine.

[0021] Exposure of the alcohol **(B)** (in which the Pro-N10-nitrogen is generally protected as carbamate) to tetrapropylammonium perruthenate (TPAP)/N-methylmorpholine N-oxide (NMO) over A4 sieves results in oxidation accompanied by spontaneous B-ring closure to afford the desired product. The TPAP/NMO oxidation procedure is found to be particularly convenient for small scale reactions while the use of DMSO-based oxidation methods, particularly Swern

oxidation, proves superior for larger scale work (e.g. > 1 g).

[0022] The uncyclized alcohol **(B)** may be prepared by the reaction of a nitrogen protection reagent of formula **D**, which is preferably a chloroformate or acid chloride, to a solution of the amino alcohol **C**, generally in solution, generally in the presence of a base such as pyridine (preferably 2 equivalents) at a moderate temperature (e.g. at 0°C). Under these conditions little or no O-acylation is usually observed.

[0023] The key amino alcohol **C** may be prepared by reduction of the corresponding nitro compound **E**, by choosing a method which will leave the rest of the molecule intact. Treatment of **E** with tin (II) chloride in a suitable solvent, e.g. refluxing methanol, generally affords, after the removal of the tin salts, the desired product in high yield.

[0024] Exposure of **E** to hydrazine/Raney nickel avoids the production of tin salts and may result in a higher yield of **C**, although this method is less compatible with the range of possible C and A-ring substituents. For instance, if there is C-ring unsaturation (either in the ring itself, or in R_2 or R_3), this technique may be unsuitable.

[0025] The nitro compound of formula **E** may be prepared by coupling the appropriate o-nitrobenzoyl chloride to a compound of formula **F**, e.g. in the presence of K_2CO_3 at -25°C under a N_2 atmosphere. Compounds of formula **F** can be readily prepared, for example by olefination of the ketone derived from L-transhydroxy proline. The ketone intermediate can also be exploited by conversion to the enol triflate for use in palladium mediated coupling reactions.

[0026] The o-nitrobenzoyl chloride is synthesised from the o-nitrobenzoic acid (or alkyl ester after hydrolysis) of formula **G**, which itself is prepared from the vanillic acid (or alkyl ester) derivative **H**. Many of these are commercially available and some are disclosed in Althuis, T.H. and Hess, H.J., *J. Medicinal Chem.*, 20(1), 146-266 (1977).

Alternative Cyclisation (Scheme 2)

[0027]
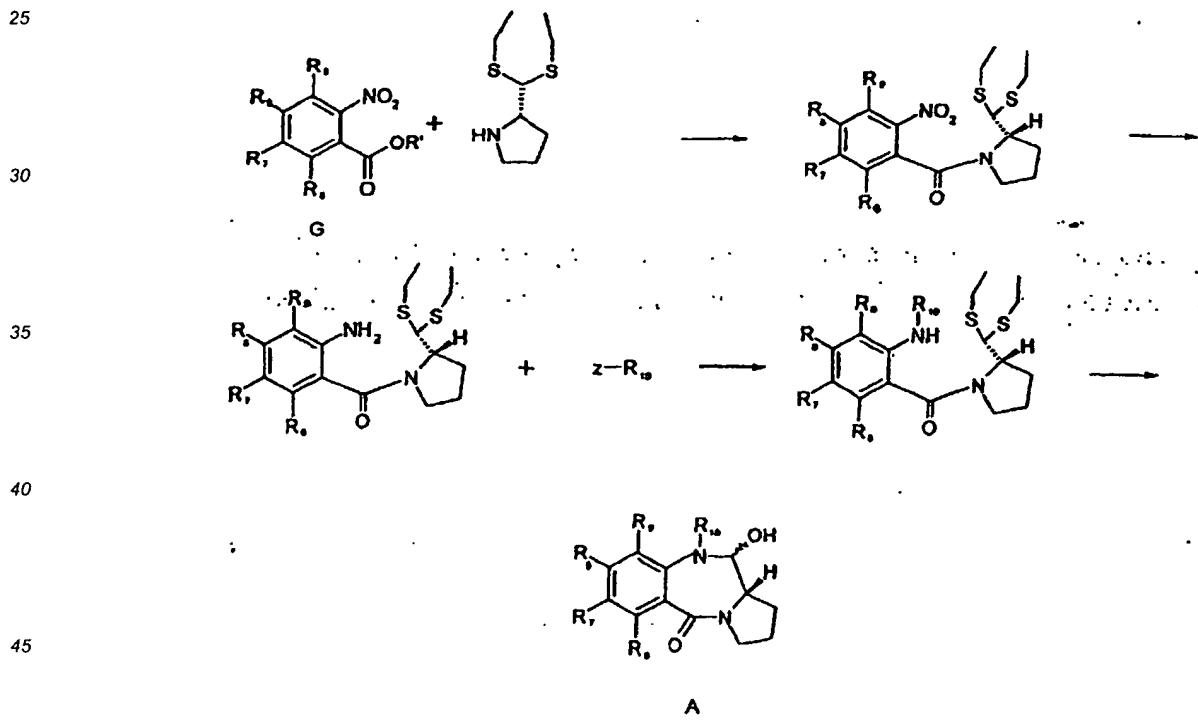
Scheme 2

[0028] In scheme 1, the final or penultimate step was an oxidative cyclisation. An alternative, using thioacetal coupling, is shown in scheme 2. Mercury-mediated unmasking causes cyclisation to the protected PBD compound **(A)**.

[0029] The thioacetal compound may be prepared as shown in scheme 2: the thioacetal protected C-ring [prepared via a literature method: Langley, D.R. & Thurston, D.E., *J. Organic Chemistry,* 52, 91-97 (1987)] is coupled to the o-nitrobenzoic acid (or alkyl ester after hydrolysis) (G) using a literature procedure. The resulting nitro compound cannot be reduced by hydrogenation, because of the thioacetal group, so the tin(II) chloride method is used to afford the amine. This is then N-protected, e.g., by reaction with a chloroformate or acid chloride, such as 2,2,2-trichloroethylchlorofor-

mate.

[0030] Acetal-containing C-rings can be used as an alternative in this type of route with deprotection involving other methods, including the use of acidic conditions.

Dimer Synthesis (Scheme 3)

[0031]
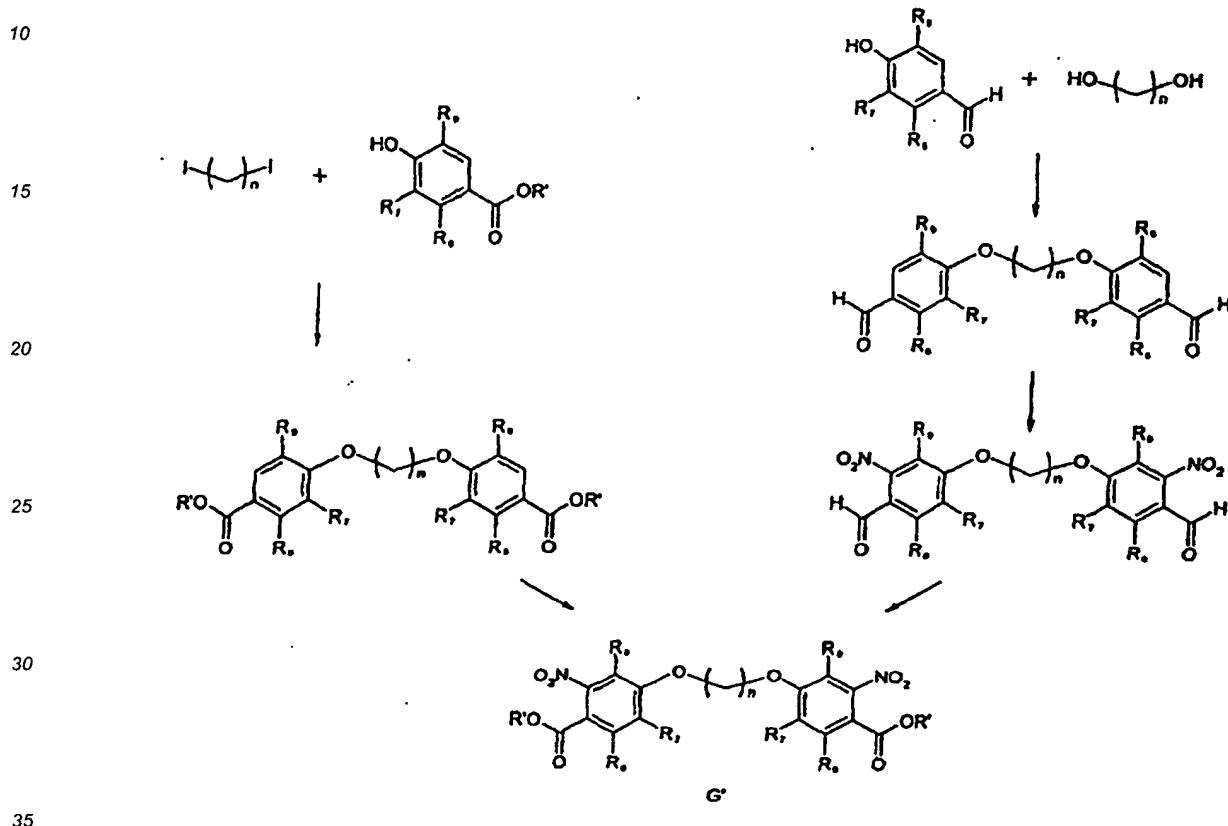
Scheme 3

[0032] PBD dimers of the invention may be synthesized using the strategy developed for the synthesis of the protected PBD monomers. The synthesis routes illustrated in scheme 3 show compounds when the dimer linkage is of the formula -O-$(CH_2)_n$-O-The step of dimer formation is normally carried out to form a bis(nitro acid) **G'**. This compound can then be treated as compound **G** in either scheme 1 or scheme 2 above.

[0033] The bis(nitro acid) **G'** may be obtained by nitrating (e.g. using 70% nitric acid) the bis(carboxylic acid). This can be synthesised by alkylation of two equivalents of the relevant benzoic acid with the appropriate diiodoalkane under basic conditions. Many benzoic acids are commercially available and others can be synthesised by conventional methods. Alternatively, the relevant benzoic acid esters can be joined together by a Mitsunobo etherification with an appropriate alkanediol, followed by nitration, and then hydrolysis (not illustrated).

[0034] An alternative synthesis of the bis(nitro acid) involves oxidation of the bis(nitro aldehyde), e.g. with potassium permanganate. This can be obtained in turn by direct nitration of the bis(aldehyde), e.g. with 70% HNO_3. Finally, the bis(aldehyde) can be obtained via the Mitsunobu etherification of two equivalents of the benzoic aldehyde with the appropriate alkanediol.

[0035] An alternative synthesis approach to those detailed above is to protect the pro N10 position on the component which will form the A-ring, before joining the component which will form the C-ring.

Preferred Synthesis Strategies for Compounds

[0036] The synthesis route of scheme 1 is generally applicable to compounds of the present invention.

[0037] C2-unsaturated PBDs may be synthesised from their N10-carbamate protected precursors. Typically, palladium catalysed removal of an allyl carbamate may be used to generate the N10-C11 imine without affecting the key C2-unsaturation. Alternatively, cadmium-lead couple may be employed to cleave an N10-2,2,2-trichloroethyl carbamate from the protected PBD.

[0038] The reduction of the nitro-compound **E** as shown in scheme 1 with tin (II) chloride maintains the C2-unsaturation, although isolating the aniline **C** from the tin salts can be problematic.

[0039] The compound of formula **F** may be used in its TBDMS protected form, and therefore a deprotection step has to be included to produce the amino-alcohol compound **E**.

[0040] The TBDMS ether of type **E**, which is the product of the coupling of the TBDMS protected compound with the appropriate o-nitrobenzoyl chloride, can be treated with AcOH:THF:H_2O (3:1:1). TBAF was found to be unsuitable for this transformation due to the rapid degradation of reaction products.

[0041] C-ring providing compounds **F(II)** can be obtained as shown in scheme 4.

Scheme 4
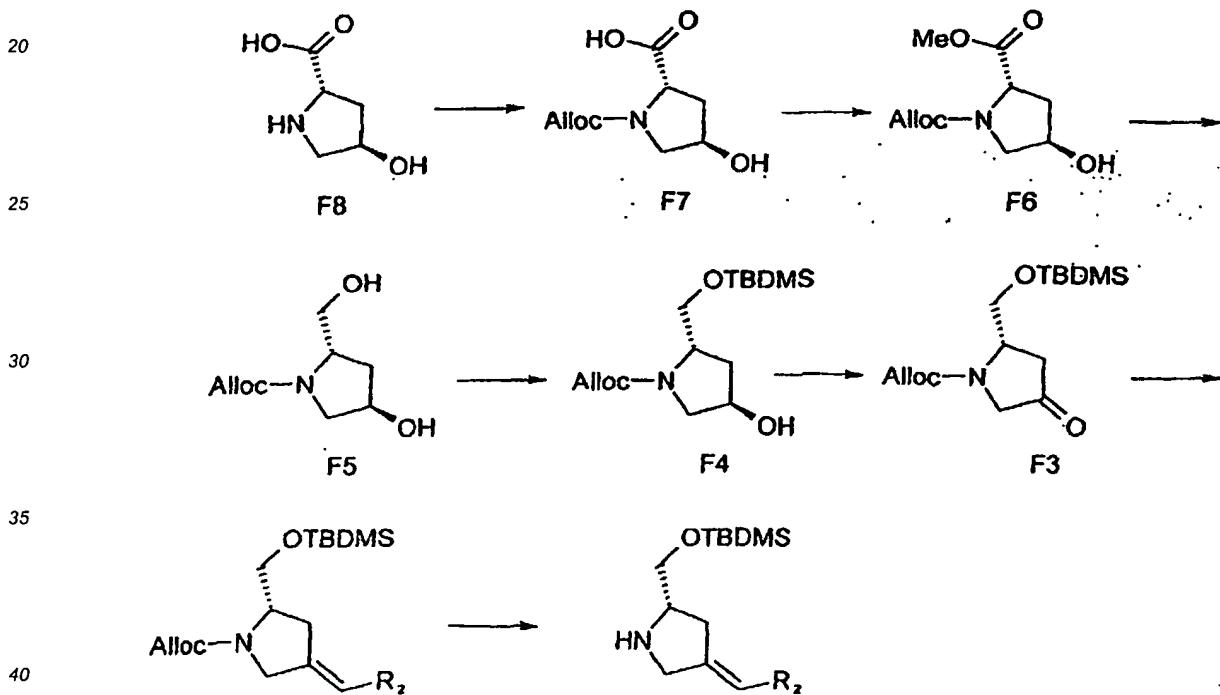
[0042] Commercially available *trans*-4-hydroxy-L-proline **F8** can be N-alloc protected to give the allyl carbamate **F7** which can then be esterified using standard conditions. Hydride reduction of the ester **F6** furnishes the diol **F5**. Selective TBDMS protection of the diol gives a silyl ether **F4**, which can then be oxidised, using either Swern or TPAP oxidation, to provide the ketone **F3**.

[0043] The C2-olefinic functionality present in **F2** may be introduced by performing the Wittig reaction on ketone **F3**. Palladium-mediated cleavage of the N-alloc protecting group (Dangles O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A.; *J. Org. Chem.* **1987**, *52*, 4984) yields compound **F(II)**.

Alternative route to compound C

[0044]
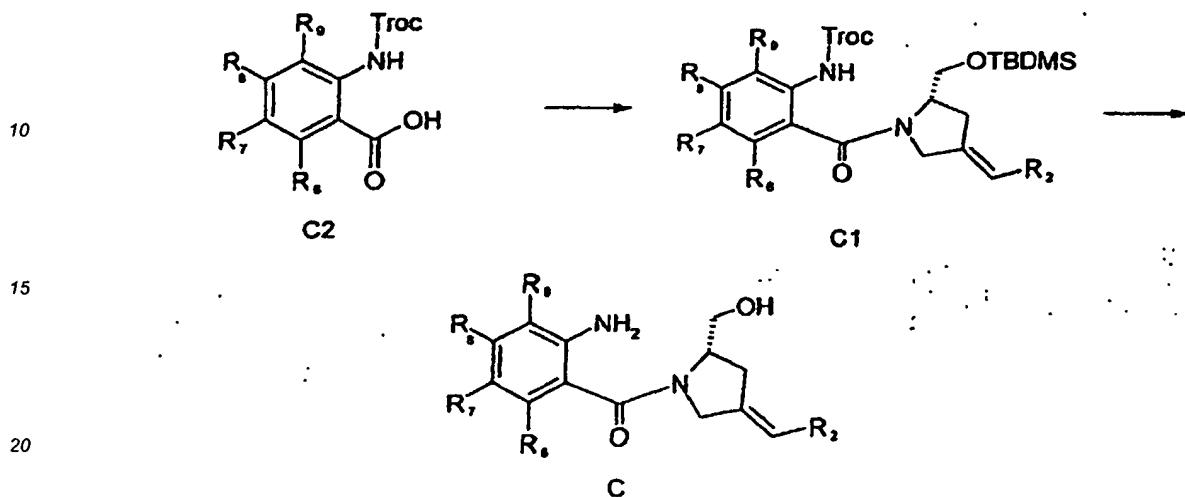
Scheme 5

An alternative route to compound C has been developed (**Scheme 5**). The amide of formula **C1** may be synthesised by forming the acid chloride of an N-Troc protected anthranilic acid of type **C2**. Interestingly, N-Troc anthranilic acids do not generate isatoic anhydrides, thus enabling amide formation reactions with amines of type **F(II)**. Simultaneous TBAF-mediated cleavage of the 2,2,2- trichloroethyl carbamate and TBDMS groups from **C1** may provide the key amino-alcohol **C**.

Alternative Route

[0045]
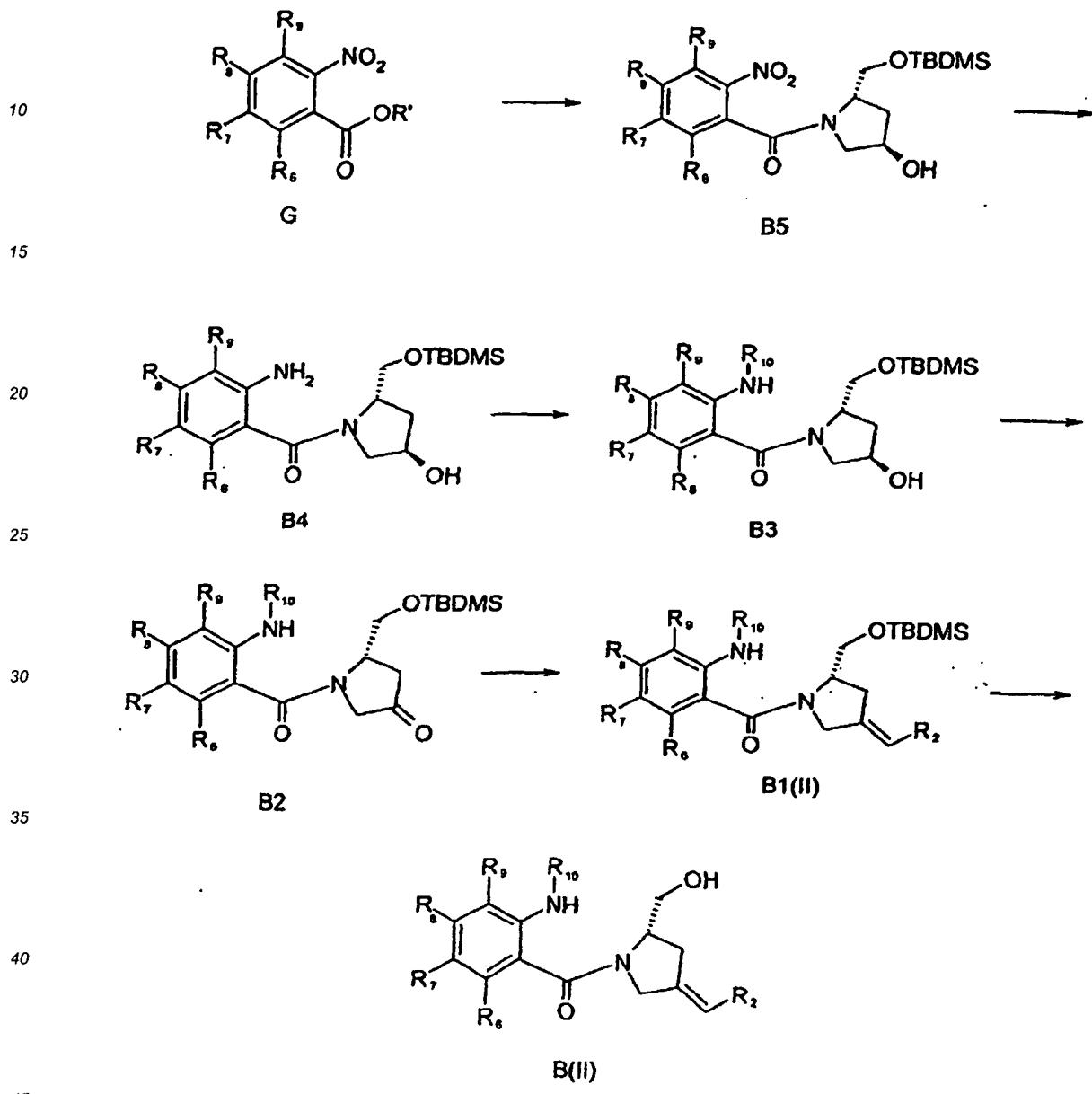
Scheme 6

[0046] A more linear synthetic route to compound **B** of scheme 1 has been developed which enables larger scale production of the C2-unsaturated PBDs, and is shown in scheme 6. TBAF-mediated cleavage of the TBDMS group may be used to produce **B(II)** from **B1(II)**. The key C2-unsaturation present in **B1(II)** may be introduced by performing the Wittig olefination reaction on a ketone of type **B2**. Swern oxidation of the secondary alcohol **B3** may be used to furnish the ketone **B2**. The carbamate protected aniline **B3** may be prepared from the nitro compound **B5** in two steps. Firstly, the nitro group may be reduced to the aniline by employing the Raney nickel/hydrazine method because a compound of type **B5** lacks C2-unsaturation. This method is more advantageous than the tin (II) chloride procedure since the product is easier to isolate. The aniline **B4** may then be N-carbamate protected in high yield without significant

carbonate formation at the C2 oxygen.

[0047] An amide of type **B5** may be synthesised by coupling an acid chloride of type G to the key amine **KEC5** (Scheme 7).
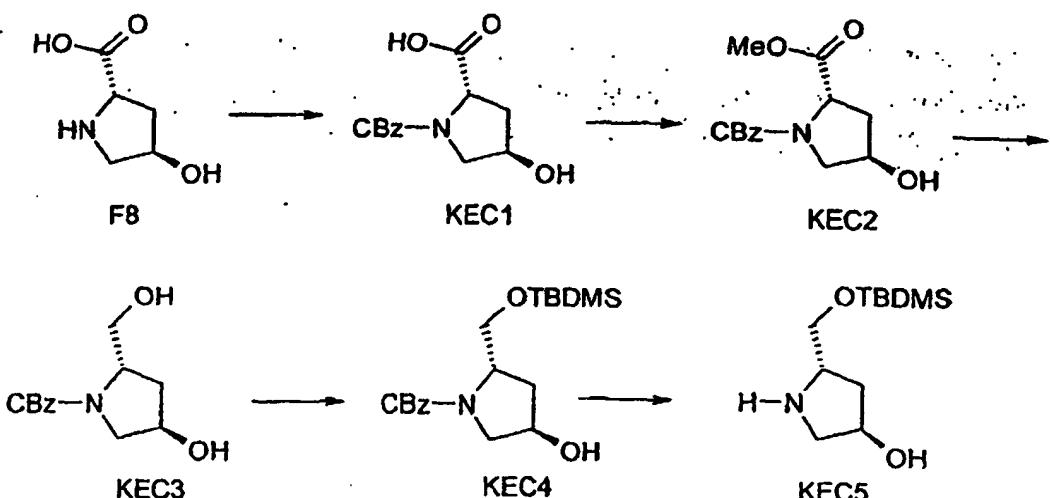
Scheme 7

[0048] Overall, this route has several advantages over the previous route which results in the larger scale production of the C2/C3-endo-unsaturated PBDs. Firstly, catalytic hydrogenation of **KEC4** allows large scale preparation of key intermediate **KEC5**. Secondly, this more efficient nitro reduction step may be carried out on an intermediate devoid of C2-unsaturation. Importantly, the double-bond migration observed during the Horner-Emmons reaction is spontaneous, so excess sodium hydride is not necessary. This double-bond migration has also been observed by other workers (Leimgruber, W.; Batcho, A. D.; Czajkowski, R. C. *J. Am. Chem. Soc.* **1968**, *90,* 5641).

[0049] Parr-hydrogenation of **KEC4**, in order to cleave the Cbz protecting group, allowed the large scale synthesis of the key amino intermediate **KEC5**. The TBDMS ether **KEC4** was prepared by selective silylation of the primary alcohol **KEC3**, which was achieved using DBU as a silyl transfer agent. The diol **KEC3** was obtained from hydride reduction of ester **KEC2** which in turn was synthesised from carboxylic acid **KEC1**. N-Cbz protection of *trans*-4-hydroxy-L-proline **(F4)** was achieved by adopting a procedure reported in the literature (Bridges, R. J.; Stanley, M. S.; Anderson, M. W.; Cotman, C. W.; Chamberlain, R. A. *J. Med. Chem.* **1991**, *34,* 717).

[0050] In dimer synthesis, the routes set out above may be used in preference to those set out in the overall synthetic strategies. In particular, the nitrogen-protecting group may advantageously be a carbamate, as protecting groups of this type may be removed in the final step by a variety of methods which, in general, do not affect the key C2-unsaturation.

General Experimental Methods

[0051] Melting points (mp) were determined on a Gallenkamp P1384 digital melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded using a Perkin-Elmer 297 spectrophotometer. ^{1}H- and ^{13}C- NMR spectra were recorded on a Jeol GSX 270 MHZ FT-NMR spectrometer operating at 20°C +/-1°C. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (TMS). Spin multiplicities are described as: s (singlet), bs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), p (pentuplet) or m (multiplet). Mass spectra (MS) were recorded using a Jeol JMS-DX 303 GC Mass Spectrometer (EI mode: 70eV, source 117-147°C). Accurate molecular masses (HRMS) were determined by peak matching using perfluorokerosene (PFK) as an internal mass marker, and FAB mass spectra were obtained from a glycerol/thioglycerol/ trifluoroacetic acid (1:1:0.1) matrix with a source temperature of 180°C. Optical rotations at the Na-D line were obtained at ambient temperature using a Perkin-Elmer 141 Polarimeter. Analytical results were generally within +/-0.2% of the theoretical values. Flash chromatography was performed using Aldrich flash chromatography "Silica Gel-60" (E. Merck, 230-400 mesh). Thin-layer chromatography (TLC) was performed using GF$_{254}$ silica gel (with fluorescent indicator) on glass plates. All solvents and reagents, unless otherwise stated, were supplied by the Aldrich Chemical Company Ltd. and were used as supplied without further purification. Anhydrous solvents were prepared by distillation under a dry nitrogen atmosphere in the presence

of an appropriate drying agent, and were stored over 4Å molecular sieves or sodium wire. Petroleum ether refers to the fraction boiling at 40-60°C.

Examples

Example 1: Synthesis of the PBD Dimer SJG-136 (UP2001) (see Figure 1)

[0052]
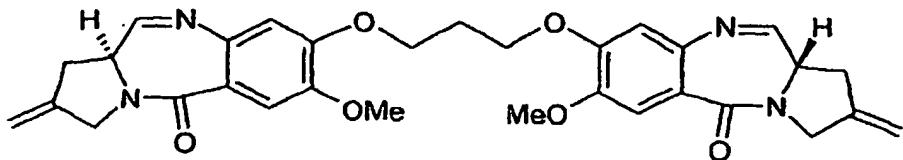
(S)-N-(Allyloxycarbonyl) -2- (tert-butyldiethylsilyloxymethyl)-4-methylidenepyrrolidine (57)

[0053] Potassium *tert*-butoxide (41.0 mL of a 0.5 M solution in THF, 20.5 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (7.29 g, 20.4 mmol) in THF (20 mL) at 0°C (ice/acetone) under nitrogen. After stirring for 2 hours at 0°C, a solution of the ketone 16 (example 1(b)) (3.20 g, 10.2 mmol) in THF (10 mL) was added dropwise and the mixture allowed to warm to room temperature. After stirring for a further 30 minutes the reaction mixture was diluted with EtOAc (150 mL) and water (150 mL) and the organic layer separated, washed with brine, dried (MgSO$_4$), filtered and evaporated *in vacuo* to give a yellow oil in which crystals (TPO) formed upon standing in the freezer. Purification by flash chromatography (5% EtOAc/Petroleum Ether) isolated the pure olefin 57 as a colourless oil (2.76 g, 87%): [α]^{21}$_D$ = -22.2 ° (c = 0.25, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) (Rotamers) δ 6.02-5.87 (m, 1H, NCO$_2$CH$_2$C*H*=CH$_2$), 5.31 (ddd, 1H, J = 1.65, 3.11, 17.20 Hz, NCO$_2$CH$_2$CH=C*H*$_2$), 5.21 (dd, 1H, J = 1.46, 10.40 Hz, NCO$_2$CH$_2$CH=C*H*$_2$), 4.99-4.61 (m, 2H, NCH$_2$C=C*H*$_2$), 4.60 (d, 2H, J = 4.94 Hz, MCO$_2$C*H*$_2$CH=CH$_2$), 4.19-3.98 (m, 2H, NCHC*H*$_2$OTBDMS), 3.93-3.87 (m, 1H, NC*H*CH$_2$OTBDMS), 3.66-3.42 (m, 2H, NC*H*$_2$C=CH$_2$), 2.80-2.56 (m, 2H, NCH$_2$C=CH$_2$C*H*$_2$), 0.87 (s, 9H, SiC(C*H*$_3$)$_3$), 0.03-0.02 (m, 6H, Si(C*H*$_3$)$_2$); ^{13}C NMR (67.8 MHz, CDCl$_3$) (Rotamers) δ 154.4 (N*C*=O), 145.1 and 144.1 (NCH$_2$*C*=CH$_2$), 133.1 (NCO$_2$CH$_2$*C*H=CH$_2$), 117.5 and 117.2 (NCO$_2$CH$_2$CH=*C*H$_2$), 107.5 and 106.9 (NCH$_2$C=*C*H$_2$), 65.8 and 65.6 (NCO$_2$*C*H$_2$CH=CH$_2$), 63.7 and 63.1 (NCH*C*H$_2$OTBDMS), 58.7 and 58.3 (N*C*HCH$_2$OTBDMS), 51.1 (N*C*H$_2$C=CH$_2$), 34.9 and 34.2 (NCH$_2$C=CH$_2$*C*H$_2$), 25.8 (SiC(*C*H$_3$)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), -5.5 (Si(*C*H$_3$)$_2$); MS (CI), m/z (relative intensity) 312 (M$^{+·}$ + 1, 82), 296 (9), 279 (5), 255 (20), 254 (M-OC$_3$H$_5$ or M-^{t}Bu, 100), 168 (8), 122 (14); IR (Neat) 3083 (C=CH$_2$), 2954, 2847, 1709 (NC=O), 1533, 1467, 1404 (SiCH$_3$), 1360, 1310, 1252 (SiCH$_3$), 1207, 1174, 1103, 1076, 1006, 836, 776, 680 cm^{-1}.

(2S)-2-(tert-butyldimethylsilyloxymethyl)-4-methylidenepyrrolidine (58)

[0054] A catalytic amount of PdCl$_2$(PPh$_3$)$_2$ (92 mg, 0.131 mmol) was added to a solution of the allyl carbamate 57 (1.0 g, 3.22 mmol) and H$_2$O (0.34 mL, 18.9 mmol) in CH$_2$Cl$_2$ (30 mL). After 5 minutes stirring at room temperature, Bu$_3$SnH (0.96 mL, 1.04 g, 3.57 mmol) was added rapidly in one portion. A slightly exothermic reaction with vigorous gas evolution immediately ensued. The mixture was stirred for 16 hours at room temperature under nitrogen at which point TLC (50% EtOAc/Petroleum Ether) revealed the formation of amine. After diluting with CH$_2$Cl$_2$ (30 mL), the organic solution was dried (MgSO$_4$), filtered and evaporated *in vacuo* to give an orange oil which was purified by flash chromatography (50-100% EtOAc/Petroleum Ether) to afford the amine 58 as a slightly orange oil (0.56 g, 77%): [α]^{21}$_D$ = -3.9 ° (c = 5.0, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) δ 4.93 (t, 1H, J = 2.02 Hz, NCH$_2$C=C*H*$_2$), 4.90 (t, 1H, J = 2.02 Hz, NCH$_2$C=C*H*$_2$), 3.68-3.46 (m, 4H, NCHC*H*$_2$OTBDMS and NC*H*$_2$C=CH$_2$), 3.30-3.21 (m, 1H, NC*H*CH$_2$OTBDMS), 2.53-2.41 (m, 2H, NCH$_2$C=CH$_2$C*H*$_2$ and N*H*), 2.26-2.17 (m, 1H, NCH$_2$C=CH$_2$C*H*$_2$), 0.90 (s, 9H, SiC(C*H*$_3$)$_3$), 0.06 (s, 6H, Si(C*H*$_3$)$_2$); ^{13}C NMR (67.8 MHz, CDCl$_3$) δ 150.0 (NCH$_2$*C*=CH$_2$), 104.7 (NCH$_2$C=*C*H$_2$), 64.7 (NCH*C*H$_2$OTBDMS), 60.5 (N*C*HCH$_2$OTBDMS), 51.3 (N*C*H$_2$C=CH$_2$), 34.9 (NCH$_2$C=CH$_2$*C*H$_2$), 25.9 (SiC(*C*H$_3$)$_3$), 18.3 (Si*C*(CH$_3$)$_3$), -5.4 (Si(*C*H$_3$)$_2$); MS (EI), m/z (relative intensity) 227 (M$^{+·}$, 8), 212 (6), 170 (M-^{t}Bu, 36), 96 (8), 82 (M-CH$_2$OTBDMS, 100), 75 (11); IR (Neat) 3550-3100 (br, NH), 3074 (C=CH$_2$), 2929, 2857, 1664 (C=C), 1472, 1424, 1391, 1380, 1361, 1255, 1190, 1101, 1006, 939, 880, 838, 777, 723, 668 cm^{-1}.

1', 3'-Bis(4-carboxy-2-methoxyphenoxy)propane (43)

[0055] A solution of diiodopropane (8.79 g, 29.7 mmol) in THF (50 mL), was added dropwise over a period of 4 hours to a vigorously stirred solution of vanillic acid (10 g, 59.5 mmol) in THF (100 mL) and aqueous NaOH (225 mL, 0.5 M) at 65°C in the absence of light (foil-wrapped flask). After heating at reflux for 48 hours in the dark, the suspension was cooled, washed with hexane (3 x 100 mL) and the THF removed by evaporation *in vacuo*. The aqueous residue was acidified to pH 1 with conc. HCl and the resultant precipitate collected by filtration, dried and recrystallised from glacial acetic acid to afford the corresponding bis-carboxylic acid **(143)** as a white crystalline solid (9.4g, 84%). mp 238-240°C; ^{1}H-NMR (DMSO-d_6) : δ 2.23 (t, 2H, J = 6.0 Hz, **H13**), 3.80 (s, 6H, **CH$_3$O**), 4.20 (t, 4H, J = 6.0 Hz, **H12**), 7.09 (d, 2H, J = 8.4 Hz, **H10**), 7.45 (d, 2H, J = 1.8 Hz, **H6**) 7.54 (dd, 2H, J = 8.4 Hz, 1.8 Hz, **H9**), 12.76 (bs, 2H, **CO$_2$H**); ^{13}C-NMR (DMSO-d_6) δ 28.4 (**C13**), 55.4 (**CH$_3$O**), 64.8 (**C12**), 111.9 (**C9**), 112.0 (**C6**), 122.9 (**C10**), 123.0 (**Q**), 148.3 (**Q**), 151.6 (**Q**), 167.0 (**C=O**). IR (KBr): ν = 3600-2000, 1680 (C=O), 1600 (C=C), 1515, 1465, 1430, 1345, 1310, 1270, 1225 (C-O-C), 1180, 1140, 1115, 1030, 990, 970, 950, 925, 875, 850, 825, 765, 725, 645 cm^{-1}. MS (EI): *m/z* (relative intensity) 376 (M$^{+\cdot}$, 28), 360 (3), 249 (2), 209 (45), 165 (29), 153 (16), 151 (19), 137 (19), 121 (7), 78 (15), 44 (100); HRMS: Calcd for $C_{19}H_{20}O_8$ = 376.1158 found 376.1168.

1',3'-Bis (4-carboxy-2-methoxy-5-nitrophenoxy)propane (44)

[0056] The diacid **43** (2.0 g, 5.30 mmol) was added portionwise to conc. HNO$_3$ (40 mL) at -10°C and stirred to room temperature over 12 h. The reaction mixture was poured on to ice (400 mL) and the resulting precipitate collected by filtration, washed with ether (3 x 50 mL) and dried to afford the nitro acid **(121)** as a yellow solid (1.73 g, 70%). m.p. 243-246°C. ^{1}H-NMR (DMSO-d_6): δ 2.25 (t, 2H, J = 5.9 Hz, **H13**), 3.90 (s, 6H, **CH$_3$O**), 4.27 (t, 4H, J = 5.9 Hz, **H12**), 7.29 (s, 2H, **H6**), 7.62 (s, 2H, **H9**), 13.6 (bs, 2H, **CO$_2$H**). ^{13}C-NMR (DMSO-d_6) δ 28.0 (**C13**), 56.3 (**CH$_3$O**), 65.7 (**C12**), 108.0 (**C9**), 111.2 (**C6**), 121.1 (**C5**), 141.3 (**Q**), 149.1 (**C8**), 151.7 (**Q**), 165.9 (**C=O**). IR (KBr): ν = 3620-2280, 1700 (C=O), 1595 (C=C), 1570, 1515 (NO$_2$), 1460, 1415, 1350 (NO$_2$), 1270, 1210, 1180, 1135, 1045, 930, 880, 810, 750, 730, 645 cm^{-1}. MS (EI): *m/z* (relative intensity) 467 (MH$^{+\cdot}$, 1), 450 (1), 436 (3), 423 (8), 378 (4), 268 (1), 255 (4), 236 (4), 210 (7), 194 (2), 182 (7), 164 (14), 153 (2), 123 (3), 91 (6), 77 (3), 55 (5), 44 (100). HRMS (EI) *m/z* calcd for $C_{19}H_{18}N_2O_{12}$ = 466.0860 found 466.0871.

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-nitro-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(*tert*-butyldimethylsilyloxymethyl)-4-methylidenepyrrolidine] (75)

[0057] A catalytic amount of DMF (2 drops) was added to a solution of the dimer acid **44** (0.66 g, 1.42 mmol) and oxalyl chloride (0.31 mL, 0.45 g, 3.55 mmol) in THF (12 mL). The reaction mixture was stirred for 16 hours under nitrogen, concentrated *in vacuo*, and redissolved in THF (10 mL). The resulting solution of *bis*-acid chloride was added dropwise to the amine **58** (0.65 g, 2.86 mmol), H$_2$O (0.84 mL) and TEA (0.83 mL, 0.60 g, 5.93 mmol) in THF (2 mL) at 0°C (ice/acetone) under nitrogen. The reaction mixture was allowed to warm to room temperature and stirred for a further 2 hours at which time TLC (EtOAc) revealed reaction completion. After removal of the THF by evaporation *in vacuo*, the residue was partitioned between H$_2$O (100 mL) and EtOAc (100 mL). The aqueous layer was washed with EtOAc (3 X 50 mL), and the combined organic layers washed with saturated NH$_4$Cl (100 mL), brine (100 mL), dried (MgSO$_4$), filtered and concentrated *in vacuo* to give the crude product as a dark orange oil. Purification by flash chromatography (50% EtOAc/Petroleum Ether) afforded the pure amide **75** as a pale yellow glass (0.93 g, 74%): $[\alpha]^{21}_D$ = -51.1° (*c* = 0.08, CHCl$_3$); ^{1}H NMR (270 MHz, CDCl$_3$) (Rotamers) δ 7.77 and 7.74 (s x 2, 2H$_{arom}$), 6.81 and 6.76 (s x 2, 2H$_{arom}$), 5.09-4.83 (m, 4H, NCH$_2$C=CH$_2$), 4.60 (m, 2H, NC*H*CH$_2$OTBDMS), 4.35-4.31 (m, 4H, OC*H$_2$*CH$_2$CH$_2$O), 4.08-3.74 (m, 14H, NCHC*H$_2$*OTBDMS, NC*H$_2$*C=CH$_2$ and OCH$_3$), 2.72-2.45 (m, 6H, NCH$_2$C=CH$_2$C*H$_2$* and OCH$_2$C*H$_2$*CH$_2$O), 0.91 and 0.79 (s x 2, 18H, SiC(CH$_3$)$_3$), 0.09, -0.09, and -0.12 (s x 3, 12H, Si(CH$_3$)$_2$); ^{13}C NMR (67.8 MHz, CDCl$_3$) (Rotamers) δ 166.2 (NC=O), 154.7 and 154.5 (*C*$_{quat}$), 148.4 and 148.2 (*C*$_{quat}$), 144.1 and 143.2 (*C*$_{quat}$), 137.2 (*C*$_{quat}$), 128.2 and 127.4 (*C*$_{quat}$), 110.1 and 108.6 (*C*-H$_{arom}$), 109.1 and 108.3 (*C*-H$_{arom}$), 107.5 (NCH$_2$C=*C*H$_2$), 65.7 and 65.5 (OCH$_2$CH$_2$CH$_2$O), 63.9 and 62.6 (NCHCH$_2$OTBDMS), 60.2 (NCHCH$_2$OTBDMS), 58.1 and 56.6 (OCH$_3$), 52.8 and 50.5 (NCH$_2$C=CH$_2$), 35.0 and 33.9 (NCH$_2$C=CH$_2$CH$_2$), 30.8 and 28.6 (OCH$_2$CH$_2$CH$_2$O), 25.8 and 25.7 (SiC (CH$_3$)$_3$), 18.2 (Si*C*(CH$_3$)$_3$), -5.5 and -5.6 (Si(CH$_3$)$_2$); MS (EI), *m/z* (relative intensity) 885 (M$^{+\cdot}$, 7), 828 (M-^{t}Bu, 100), 740 (M-CH$_2$OTBDMS, 20), 603 (3), 479 (26), 391 (27), 385 (25), 301 (7), 365 (10), 310 (14), 226 (8), 222 (13), 170 (21), 168 (61), 82 (39), 75 (92); IR (NUJOL®) 2923, 2853, 2360, 1647, 1587, 1523 (NO$_2$), 1461, 1429, 1371, 1336 (NO$_2$), 1277, 1217, 1114, 1061, 1021, 891, 836 772, 739 cm^{-1}.

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-nitro-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylidenepyrrolidine] (76)

[0058] A solution of TBAF (3.98 mL of a 1M solution in THF, 3.98 mmol) was added to the *bis*-silyl ether 75 (1.41 g, 1.59 mmol) in THF (35 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and after a further 30 minutes saturated NH_4Cl (120 mL) was added. The aqueous solution was extracted with EtOAc (3 X 80 mL), washed with brine (80 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to give a dark orange oil which was purified by flash chromatography (97% $CHCl_3$/MeOH) to provide the pure diol 76 as a light orange solid (0.98 g, 94%): $[\alpha]^{19}_D$ = -31.9 ° (*c* = 0.09, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) (Rotamers) δ 7.75 and 7.71 (s x 2, $2H_{arom}$), 6.96 and 6.84 (s x 2, $2H_{arom}$), 5.08, 5.02 and 4.88 (br s x 3, 4H, $NCH_2C=CH_2$), 4.61-4.50 (m, 2H, $NCHCH_2OH$), 4.35-4.33 (m, 4H, $OCH_2CH_2CH_2O$), 4.02-3.65 (m, 14H, $NCHCH_2OH$, $NCH_2C=CH_2$ and OCH_3), 2.88-2.43 (m, 6H, $NCH_2C=CH_2CH_2$ and $OCH_2CH_2CH_2O$); ^{13}C NMR (67.8 MHz, $CDCl_3$) (Rotamers) δ 167.9 and 166.9 (NC=O), 154.9 and 154.3 (C_{quat}), 148.4 and 148.2 (C_{quat}), 143.3 and 142.6 (C_{quat}), 137.2 and 137.0 (C_{quat}), 127.6 and 127.3 (C_{quat}), 109.1 (C-H_{arom}), 108.4 ($NCH_2C=CH_2$), 108.2 (C-H_{arom}), 65.6 and 65.4 ($OCH_2CH_2CH_2O$), 64.5 and 63.3 ($NCHCH_2OH$), 60.5 and 60.0 ($NCHCH_2OH$), 56.8 and 56.7 (OCH_3), 52.9 ($NCH_2C=CH_2$), 35.0 and 34.3 ($NCH_2C=CH_2CH_2$), 29.6 and 28.6 ($OCH_2CH_2CH_2O$); MS (FAB) (Relative Intensity) 657 (M^+ + 1, 10), 639 (M-OH, 2), 612 (1), 544 (M-$NCH_2CCH_2CH_2CHCH_2OH$, 4), 539 (1), 449 (16), 433(9), 404 (8), 236 (32), 166 (65), 151 (81), 112 (82), 82 (100); IR (NUJOL®) 3600-3200 (br, OH), 2923, 2853, 2360, 1618, 1582, 1522 (NO_2), 1459, 1408, 1375, 1335 (NO_2), 1278, 1218, 1061, 908, 810, 757 cm^{-1}.

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylidenepyrrolidine] (77)

[0059] A mixture of the diol 76 (0.98 g, 1.49 mmol) and $SnCl_2.2H_2O$ (3.36 g, 14.9 mmol) in MeOH (35 mL) was heated at reflux and the progress of the reaction monitored by TLC (90% $CHCl_3$/MeOH). After 45 minutes, the MeOH was evaporated *in vacuo* and the resulting residue was cooled (ice), and treated carefully with saturated $NaHCO_3$ (120 mL). The mixture was diluted with EtOAc (120 mL), and after 16 hours stirring at room temperature the inorganic precipitate was removed by filtration through celite. The organic layer was separated, washed with brine (100 mL), dried ($MgSO_4$), filtered and evaporated in *vacuo* to give a brown solid. Flash chromatography (95% $CHCl_3$/MeOH) afforded the pure *bis*-amine 77 as an orange solid (0.54 g, 61%): $[\alpha]^{19}_D$ = -31.8 ° (*c* = 0.30, $CHCl_3$); ^1H NMR (270 MHz, $CDCl_3$) δ 6.74 (s, $2H_{arom}$), 6.32 (s, $2H_{arom}$), 5.00 (br s, 2H, $NCH_2C=CH_2$), 4.93 (br s, 2H, $NCH_2C=CH_2$), 4.54 (br s, 2H, $NCHCH_2OH$), 4.24-4.14 (m, 4H, $OCH_2CH_2CH_2O$), 3.98-3.50 (m, 14H, $NCHCH_2OH$, $NCH_2C=CH_2$ and OCH_3), 2.76 (dd, 2H, *J* = 8.61, 15.91 Hz, $NCH_2C=CH_2CH_2$), 2.46-2.41 (m, 2H, $NCH_2C=CH_2CH_2$), 2.33-2.28 (m, 2H, $OCH_2CH_2CH_2O$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 171.0 (NC=O), 151.0 (C_{quat}), 143.5 (C_{quat}), 141.3 (C_{quat}), 140.6 (C_{quat}), 112.4 (C-H_{arom}), 111.9 (C_{quat}), 107.8 ($NCH_2C=CH_2$), 102.4 (C-H_{arom}), 65.2 ($OCH_2CH_2CH_2O$), 65.0 ($NCHCH_2OH$), 59.8 ($NCHCH_2OH$), 57.1 (OCH_3), 53.3 ($NCH_2C=CH_2$), 34.4 ($NCH_2C=CH_2CH_2$), 29.0 ($OCH_2CH_2CH_2O$); MS (FAB) (Relative Intensity) 596 (M^+, 13), 484 (M-$NCH_2CCH_2CH_2CHCH_2OH$, 14), 389 (10), 371 (29), 345 (5), 224 (8), 206 (44), 166 (100), 149 (24), 112 (39), 96 (34), 81 (28); IR (NUJOL®) 3600-3000 (br, OH), 3349 (NH_2), 2922, 2852, 2363, 1615, 1591 (NH_2), 1514, 1464, 1401, 1359, 1263, 1216, 1187, 1169, 1114, 1043, 891, 832, 761 cm^{-1}.

(2S,4R)&(2S,4S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-amino-5-methoxy-1,4-phenylene)carbonyl]]bis[2-(hydroxymethyl)-4-methylpyrrolidine] (77).

[0060] A solution of hydrazine (23 mg, 23 µL, 0.72 mmol) in MeOH (5 mL) was added dropwise to a solution of the diol 76 (95 mg, 0.145 mmol) and Raney Ni (20 mg) in MeOH (15 mL) heated at reflux. After 1 hour at reflux TLC (90% $CHCl_3$/MeOH) revealed some amine formation. The reaction mixture was treated with further Raney Ni (20 mg) and hydrazine (23 mg, 23 µL, 0.72 mmol) in MeOH (5 mL) and was heated at reflux for an additional 30 minutes at which point TLC revealed complete reaction. The reaction mixture was then treated with enough Raney Ni to decompose any remaining hydrazine and heated at reflux for a further 1.5 hours. Following cooling to room temperature the mixture was filtered through a sinter and the resulting filtrate evaporated *in vacuo*. The resulting residue was then treated with CH_2Cl_2 (30 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo* to provide the bis-amine (77) as a yellow oil (54 mg, 63%): ^1H NMR (270 MHz, $CDCl_3$) (diastereoisomers) δ 6.73 (s, $2H_{arom}$), 6.32 (s, $2H_{arom}$), 4.60-4.30 (m, 2H, $NCHCH_2OH$), 4.19 (t, 4H, *J* = 5.87 Hz, $OCH_2CH_2CH_2O$), 3.78-3.50 (m, 14H, $NCHCH_2OH$, NCH_2CHCH_3 and OCH_3), 2.40-1.55 (m, 8H, NCH_2CHCH_3, $OCH_2CH_2CH_2O$ and $NCH_2CHCH_3CH_2$), 1.00-0.95 (m, 6H, NCH_2CHCH_3); MS (EI), *m/z* (relative intensity) 600 (M^+, 16), 459 (46), 345 (16), 206 (13), 186 (17), 180 (31), 166 (37), 149 (6), 142 (76), 100 (6), 98 (13), 97 (29), 84 (81), 69 (7), 55 (100).

(2S)-1,1'-[[(Propane-1,3-diyl)dioxy]bis[(2-allyloxycarbonylamino-5-methoxy-1,4-phenylene)carbonyl]]bis [2-(hydroxymethyl)-4-methylidenepyrrolidine] (78)

[0061] Pyridine (0.47 mL, 0.46 g, 5.82 mmol) was added to a stirred solution of the bis-amine 77 (0.857 g, 1.44 mmol) in CH_2Cl_2 (30 mL) at 0°C (ice/acetone). The cool mixture was then treated dropwise with a solution of allyl chloroformate (0.33 mL, 0.38 g, 3.15 mmol) in CH_2Cl_2 (10 mL). After 2.5 hours stirring at room temperature, the mixture was diluted with CH_2Cl_2 (60 mL), washed with 1N HCl (2 X 50 mL), H_2O (80 mL), brine (80 mL), dried ($MgSO_4$), filtered and evaporated *in vacuo*. The crude residue was purified by flash chromatography (70-100% EtOAc/Petroleum Ether) to afford the allyl carbamate compound 78 as a slightly orange glass (0.548 g, 50%): ^1H NMR (270 MHz, $CDCl_3$) δ 8.58 (br s, 2H, NH), 7.56 (s, 2H_{arom}), 6.78 (s, 2H_{arom}), 6.03-5.88 (m, 2H, $NCO_2CH_2CH=CH_2$), 5.39-5.21 (m, 4H, $NCO_2CH_2CH=CH_2$), 5.00 (br s, 2H, $NCH_2C=CH_2$), 4.93 (br s, 2H, $NCH_2C=CH_2$), 4.70-4.57 (m, 4H, $NCO_2CH_2CH=CH_2$), 4.30-4.25 (m, 4H, $OCH_2CH_2CH_2O$), 4.17-3.90 (m, 8H, $NCHCH_2OH$ and $NCH_2C=CH_2$), 3.81-3.54 (m, 8H, $NCHCH_2OH$ and OCH_3), 2.76 (dd, 2H, $J = 8.52$, 15.85 Hz, $NCH_2C=CH_2CH_2$), 2.49-2.44 (m, 2H, $NCH_2C=CH_2CH_2$), 2.36-2.28 (m, 2H, $OCH_2CH_2CH_2O$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 170.3 (NC=O_{amide}), 153.8 (NC=$O_{carbamate}$), 150.5 (C_{quat}), 144.8 (C_{quat}), 143.1 (C_{quat}), 132.5 ($NCO_2CH_2CH=CH_2$), 130.7 (C_{quat}), 118.1 ($NCO_2CH_2CH=CH_2$), 116.8 (C_{quat}), 110.9 (C-H_{arom}), 108.1 ($NCH_2C=CH_2$), 106.9 (C-H_{arom}), 65.7 ($NCO_2CH_2CH=CH_2$), 65.4 ($OCH_2CH_2CH_2O$), 65.1 ($NCHCH_2OH$), 59.8 ($NCHCH_2OH$), 56.5 (OCH_3), 53.9 ($NCH_2C=CH_2$), 34.2 ($NCH_2C=CH_2CH_2$), 29.7 and 29.2 ($OCH_2CH_2CH_2O$); MS (FAB) (Relative Intensity) 765 (M^+· + 1, 10), 652 ($M-NCH_2CCH_2CH_2CHCH_2OH$, 32), 594 (4), 539 (2), 481 (51), 441 (31), 290 (3), 249 (13), 232 (38), 192 (83), 166 (49), 149 (32), 114 (100).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11S,11aS)-10-(allyloxycarbonyl)-11-hydroxy-7-methoxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4]benzodiazepin-5-one] (79)

[0062] A solution of the *bis*-alloc compound 78 (150 mg, 0.196 mmol) in CH_2Cl_2/CH_3CN (12 mL, 3:1) was treated with 4 Å powdered molecular sieves (0.2 g) and NMO (70 mg, 0.598 mmol). After 15 minutes stirring at room temperature, TPAP (7 mg, 19.9 µmol) was added and stirring continued for a further 2 hours at which time TLC (95% $CHCl_3$/MeOH) indicated formation, of the fully cyclised product along with the presumed semi-cyclised product 79, and unreacted starting material 78 present in the reaction mixture. The mixture was then treated with a further quantity of NMO (35 mg, 0.299 mmol) and TPAP (3.5 mg, 9.96 µmol), and allowed to stir for a further 0.5 hours when TLC revealed reaction completion. The solvent was evaporated *in vacuo* and the black residue was subjected to flash chromatography (98% $CHCl_3$/MeOH) to provide the pure protected carbinolamine 79 as a white solid (47 mg, 32%): ^1H NMR (270 MHz, $CDCl_3$) δ 7.23 (s, 2H_{arom}), 6.74 (s, 2H_{arom}), 5.90-5.65 (m, 2H, $NCO_2CH_2CH=CH_2$), 5.57 (d, 2H, $J = 8.24$ Hz, NCHCHOH), 5.26-5.07 (m, 8H, $NCH_2C=CH_2$ and $NCO_2CH_2CH=CH_2$), 4.67-4.10 (m, 14H, $NCO_2CH_2CH=CH_2$, $NCH_2C=CH_2$, $OCH_2CH_2CH_2O$ and OH), 3.89 (s, 6H, OCH_3), 3.63 (m, 2H, NCHCHOH), 2.91 (dd, 2H, $J = 8.79$, 15.76 Hz, $NCH_2C=CH_2CH_2$), 2.68 (d, 2H, $J = 16.10$ Hz, $NCH_2C=CH_2CH_2$), 2.42-2.24 (m, 2H, $OCH_2CH_2CH_2O$); ^{13}C NMR (67.8 MHz, $CDCl_3$) δ 166.7 (NC=O_{amide}), 150.1 (C_{quat}), 149.0 (C_{quat}), 141.7 (C_{quat}), 131.7 ($NCO_2CH_2CH=CH_2$), 130.6 (C_{quat}), 128.9 (C_{quat}), 128.8 (C_{quat}), 118.3 ($NCO_2CH_2CH=CH_2$), 114.7 (C-H_{arom}), 110.7 (C-H_{arom}), 109.8 ($NCH_2C=CH_2$), 85.9 (NCHCHOH), 66.9 ($NCO_2CH_2CH=CH_2$), 66.0 ($OCH_2CH_2CH_2O$), 59.7 (NCHCHOH), 56.1 (OCH_3), 50.7 ($NCH_2C=CH_2$), 35.0 ($NCH_2C=CH_2CH_2$), 29.7 and 29.1 ($OCH_2CH_2CH_2O$); MS (FAB) (Relative Intensity) 743 (M^+· - 17, 16), 725 (17), 632 (13), 574 (8), 548 (13), 490 (10), 481 (9), 441 (7), 425 (6), 257 (12), 232 (20), 192 (46), 166 (52), 149 (100), 91 (59); IR (NUJOL®) 3234 (br, OH), 2923, 2853, 2361, 1707, 1604, 1515, 1464, 1410, 1377, 1302, 1267, 1205, 1163, 1120, 1045, 999, 955, 768, 722 cm^{-1}.

1,1'-[[(Propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1c][1,4] benzodiazepin-5-one] (80, SJG-136)

[0063] A catalytic amount of tetrakis(triphenylphosphine)palladium (11 mg, 9.52 µmol) was added to a stirred solution of the *bis*-alloc-carbinolamine 79 (139 mg, 0.183 mmol), triphenylphosphine (4.8 mg, 18.3 µmol) and pyrrolidine (27 mg, 0.380 mmol) in CH_2Cl_2/CH_3CN (13 mL, 10:3) at 0°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and the progress monitored by TLC (95% $CHCl_3$/MeOH). After 2 hours 15 minutes TLC revealed the reaction was complete, proceeding via the presumed half-imine product 261, to give a TLC spot which fluoresced brightly under UV. The solvent was evaporated *in vacuo* and the resulting residue subjected to flash chromatography (98% $CHCl_3$/MeOH) to give the *bis*-imine target molecule 80 (SJG-136) as a pale orange glass (78 mg, 77%) which was repeatedly evaporated *in vacuo* with $CHCl_3$ to provide the imine form: $[\alpha]^{21}_D = +357.7$ ° ($c = 0.07$, $CHCl_3$); Reverse Phase HPLC (C_4 stationary phase, 65% MeOH/H_2O mobile phase, 254 nm), Retention time = 6.27 minutes, % Peak area = 97.5%; ^1H NMR (270 MHz, $CDCl_3$) (imine form) δ 7.68 (d, 2H, J= 4.4 Hz, HC=N), 7.49 (s, 2H_{arom}), 6.85 (s, 2H_{arom}), 5.20 (s, 2H, $NCH_2C=CH_2$), 5.17 (s, 2H, $NCH_2C=CH_2$), 4.46-4.19 (m, 4H, $OCH_2CH_2CH_2O$), 3.92 (s, 6H, OCH_3), 3.89-3.68 (m, 6H, $NCH_2C=CH_2$ and NCHHC=N), 3.12 (dd, 2H, $J = 8.61$,

16.21 Hz, NCH$_2$C=CH$_2$CH_2), 2.68 (d, 2H, J = 16.30 Hz, NCH$_2$C=CH$_2$CH_2), 2.45-2.38 (m, 2H, OCH$_2$CH_2CH$_2$O); ^{13}C NMR (67.8 MHz, CDCl$_3$) (imine form) δ 164.7 (NC=O), 162.6 (HC=N), 150.7 (C_{quat}), 147.9 (C_{quat}), 141.5 (C_{quat}), 140.6 (C_{quat}), 119.8 (C_{quat}), 111.5 (C-H$_{arom}$), 110.7 (C-H$_{arom}$), 109.4 (NCH$_2$C=CH$_2$), 65.4 (OCH$_2$CH$_2$CH$_2$O), 56.1 (OCH$_3$), 53.8 (NCHHC=N), 51.4 (NCH$_2$C=CH$_2$), 35.4 (NCH$_2$C=CH$_2$$CH_2$), 28.8 (OCH$_2$$CH_2CH_2$O); MS (FAB) (Relative Intensity) (imine form) 773 (M$^+$· + 1 + (Thioglycerol adduct X 2), 3), 665 (M$^+$· + 1 + Thioglycerol adduct, 7), 557 (M$^+$· + 1, 9), 464 (3), 279 (12), 257 (5), 201 (5), 185 (43), 166 (6), 149 (12), 93 (100); IR (NUJOL®) 3600-3100 (br, OH of carbinolamine form), 2923, 2849, 1599, 1511, 1458, 1435, 1391, 1277, 1228, 1054, 1011, 870, 804, 761, 739 cm^{-1}.

Alternative Synthesis of UP2001, SJG-136 (80) (see Figures 2a and 2b)

(2S,4R)-N-(Benzoxycarbonyl)-2-carboxy-4-hydroxypyrrolidine (45)

[0064]   A solution of benzyl chloroformate (12.5 mL, 87.7 mL) in toluene (40 mL) was added to a solution of *trans*-4-hydroxy-L-proline 11 (10 g, 76.3 mmol) and NaHCO$_3$ (16 g, 190 mmol) in H$_2$O (165 mL) over a period of 15minutes. After stirring at room temperature for 12 hours the two phases were allowed to separate. The aqueous phase was washed with diethyl ether (4 x 50 mL), cooled in an ice bath, and then acidified to pH 2 with conc. HCl. The resultant product was extracted with ethyl acetate (5 x 50 mL) and the combined organic extracts were dried (MgSO$_4$) and the excess solvent evaporated *in vacuo* to afford a colourless viscous oil (20.30 g, 100%). [α]^{27}$_D$ = -565° (c 0.1, MeOH). ^{1}H NMR (CDCl$_3$): δ 2.07-2.31 (m, 3H, H1), 3.52-3.59 (m, 2H, H3), 4.43-4.53 (m, 2H, H2, H11a), 5.8 and 5.11 (s, 2H, minor and major rotamers of H6, 1:2), 6.0 (bs, 2H, OH), 7.26-7.29 and 7.32-7.34 (m, 5H, minor and major rotamers of H arom, 1:2). IR (thin film): v = 3414 (OH), 2940 (OH), 1682 (C=O), 1495, 1429, 1359 (CO$_2$$^-$), 1314, 1269, 1205, 1180, 1174, 1127, 1082, 1051, 993, 914, 866, 826, 769, 741, 697 cm^{-1}. MS (EI): m/e (relative intensity): 266 (M$^+$·, - 1), 265 (6), 220 (5), 176 (15), 130 (34), 108 (2). 91 (100), 86 (4), 68 (11). HRMS calcd. for C$_{13}$H$_{15}$NO$_5$ = 265.0950 found 265.0976

(2S,4R)-N-(Benzoxycarbonyl)-2-methyoxycarbonyl-4-hydroxyproline (46)

[0065]   A solution or (2S,4R)-N-(Benzozycarbonyl)-2-carboxy-4-hydroxypyrrolidine (45) (20.30 g, 76.3 mmol) in dry methanol (300 mL) was heated at reflux for 18 hours in the presence of a catalytic amount of conc. H$_2$SO$_4$ (2.20 mL, 7.63 mmol). The reaction mixture was allowed to cool to room temperature and neutralised with Et$_3$N (3.0 mL, 76.3 mmol). The reaction mixture was concentrated in *vacuo* and the residue redissolved in ethyl acetate (200 mL), washed with brine (1 x 50 mL), dried (MgSO$_4$) and excess solvent removed under reduced pressure to afford a colourless gum (21.17 g, 99%). [α]^{20}$_D$ = -59.4° (c 0.014, CHCl$_3$). ^{1}H NMR (CDCl$_3$) : δ 2.04-2.08 and 2.24-2.35(m, 1H, rotamers of H1, 1:1), 2.64 (bs, 1H, OH), 3.54 and 3.74 (s, 3H, rotamers of OMe, 1:1), 3.66-3.69 (m, 2H, H3), 4.47-4.50 (m, 2H, H2, H11a), 5.07-5.13 (m, 2H, H6), 7.26-7.35 (m, 5H, H arom). ^{13}C NMR (CDCl$_3$): rotamer ratio 1:1, δ 37.8 and 38.5 rotamers of (C1), 51.8 and 52.0 rotamers of (OMe), 54.1 and 54.7 rotamers of (C3), 57.4 and 57.7 rotamers of (C2), 66.9 and 67.0 rotamers of (C6), 68.6 and 69.3 rotamers of (C11a), 127.0, 127.3, 127.4, 127.7, 127.8, 128.0 and 128.1 rotamers of (C arom). IR (thin film): v = 3435 (OH), 3033, 2953 (OH), 1750 (ester), 1680 (C=O), 1586, 1542, 1498, 1422, 1357 (CO$_2$H), 1170, 1124, 1084, 1052 (C-O), 1004, 963, 916, 823, 770, 750, 699, 673 cm^{-1}. MS (FAB) m/z (relative intensity): 280 (M$^+$·, 24), 236 (20), 234 (4), 216 (8), 214 (4), 213 (2), 206 (2), 204 (7), 203 (4), 202 (10), 201 (2), 181 (5), 144 (16), 102 (23), 91 (100). HRMS calcd. for C$_{14}$H$_{17}$NO$_5$ = 279.1107 found 279.1192

(2S,4R)-N-(Benzoxycarbonyl)-2-hydroxymethyl-4-hydroxyproline (47)

[0066]   Lithium borohydride (1.57 g, 73 mmol) was added portionwise to a solution of (2S,4R)-N-(benzoxycarbonyl)-2-methyoxycarbonyl-4-hydroxyproline (46) (20.17 g, 73 mmol) in THF (350 mL) at 0°C. The reaction mixture was allowed to warm to room temperature and stir overnight. The resulting suspension was cooled to 0°C and quenched with water (2-3 mL) until effervescence ceased, at which point 2 M HCl (15 mL) was added to dissolve the precipitate. The product was extracted with ethyl acetate (3 x 150 mL) and the combined organic phases washed with brine (1 x 100 mL) and then dried (MgSO$_4$). Concentration in *vacuo* afforded a white gum (18.25 g, 100%). [α]$^{22.3}$$_D$ = -404° (C = 0.043, CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ 1.24-1.26 (m, 1H, H1), 1.73-2.08 (m, 1H, H1), 3.40-4.30 (m, 6H, H2, H3, H11, H11a), 5.06 (bs, 1H, OH), 5.09 (s, 2H, H6) 7.25-7.31 (m, 5H, H arom). ^{13}C NMR (CDCl$_3$): δ 36.7 (C1), 55.2 (C3), 58.7 (C2), 65.0 (C11), 67.0 (C6), 68.7 (C11a), 127.0, 127.5 (C arom), 127.8 (C arom), 128.2 (C arom). IR (thin film): v = 3390 (OH), 3065, 3033, 2953 (OH), 1681 (C=O carbamate), 1586, 1538, 1498, 1454, 1192, 1122, 978, 914, 862, 770, 698, 673 cm^{-1}. MS (FAB) m/z (relative intensity): 252 (M$^+$·, 58), 208 (33), 176 (5), 144 (6), 118 (8), 116 (7), 92 (13), 91 (100). HRMS calcd. for C$_{13}$H$_{17}$NO$_4$ = 251.1158 found 251.1230.

(2S,4R)-N-Benzoxycarbonyl-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (48)

[0067] t-butyldimethylsilyl chloride (5.78 g, 38.3 mmol) and 1,8-diazabicyclo[5,4,0]undec-7-ene (1.44 mL, 9.6 mmol) were added to a solution of alcohol (47) (12.51 g, 49.8 mmol) and triethylamine (7.0 mL, 49.8 mmol) in dry DCM (200 mL) which had been allowed to stir for 15minutes at room temperature. The resulting mixture was allowed to stir at room temperature for 18 hours and then diluted with ethyl acetate (300 mL). The organic phase was washed with aqueous saturated ammonium chloride (2 x 100 mL) and brine (1 x 100 mL) dried (MgSO$_4$) and the solvent removed under reduced pressure to yield a colourless viscous oil (9.84 g, 70%). [α]$^{22.3}_D$ = -263° (c 0.70, CHCl$_3$). ^{1}H NMR (CDCl$_3$) : δ -0.05 and -0.06(s, 6H, rotamers of **H1'**, **H2'**, 1:1), 0.83 and 0.85 (s, 9H, rotamers of **H3'**, **H5'**, **H6'**, 1:1), 1.95-2.22 (m, 2H, **H1,**), 2.78 (bs, 1H, **OH**), 3.44-3.68 (m, 3H, **H3**, **H11**), 3.99-4.10 (m, 1H, **H2**), 4.43-4.46 (m, 1H, **H11a**), 5.11-5.16 (m, 2H, **H6**) 7.34-7.35 (m, 5H, **H arom**) ^{13}C NMR (CDCl$_3$): rotamer ratio of 1:1, δ -5.50 **(C3, C5', C6')**, 18.15 **(C4')**, 25.83 **(C1', C2')**, 36.55 and 37.27 rotamers of **(C1)**, 55.2 and 55.7 rotamers of **(C3)**, 57.3 and 57.8 rotamers of **(C2)**, 62.8 and 63.9 rotamers of **(C11)**, 66.6 and 67.0 rotamers of (C6), 69.7 and 70.3 rotamers of **(C11a)**, 127.8 **(C arom)**, 127.9 **(C arom)**, 128.0 (C arom), 128.4 **(C arom)**, 128.5 **(C arom)**, 136.5 and 136.8 rotamers of **(C7)**, 154.9 and 155.2 rotamers of **(C5)**. IR (thin film): ν = 3415 (OH), 3066, 3034, 2953 (OH), 2930, 2884, 2857, 1703 (C=O carbamate), 1587, 1498, 1424, 1360 **(C-CH$_3$)**, 1288 (CH$_3$Si), 1255 (t-Bu), 1220, 1195 (t-Bu), 1118 (Si-O), 1057, 1003, 917, 836, 774, 751, 698, 670 cm^{-1}. MS (EI/CI) m/e (relative intensity): 366 (M$^+$, 100), 308 (14), 258 (2), 91 (2).

(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (2)

[0068] A slurry of 10% Pd/C (190 mg) in ethyl acetate (20 mL) was added to a solution of TBDMS ether (48) (1.90 g, 5.19 mmol) in ethanol (100 mL). The reaction mixture was hydrogenated (Parr apparatus) for 16 h. The catalyst was removed by vacuum filtration through Celite and excess solvent was evaporated under reduced pressure to give a yellow oil in quantitative yield (1.20 g, 100%). [α]$^{22.2}_D$ = +35.6° (c 0.042, CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ -(0.07-0.08) (m, 6H, **H1'**, **H2'**), 0.82 (s, 9H, **H3'**, **H4'**, **H5'**), 1.68-1.73 (m, 2H, **H1,**), 2.99-3.11 (m, 2H, **H11**), 3.47-3.50 (m, 3H, **H11a, H3**), 4.09 (bs, 1H, **NH or OH**), 4.32 (bs, 1H, **NH or OH**). ^{13}C NMR (CDCl$_3$) : δ -5.4 **(C3', C5', C6')**, 18.1 **(C4')**, 25.8 **(C1, C2')**, 37.4 **(C1)**, 54.6 **(C11)**, 58.1 **(C2)**, 64.6 **(C3)**, 72.2 **(C11a)**. IR (thin film): ν = 3330 (OH), 2928, 2857, 1557, 1421, 1331 (C-CH$_3$), 1249 (CH$_3$-Si), 1204 (t-Bu), 1191 (t-Bu), 1100 (Si-O), 1073, 993, 713 cm^{-1}. MS (CI) m/e (relative intensity): 232 (M$^+$, 100), 230 (13), 174 (5), 133 (6), 86 (6).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-nitro-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (49)

[0069] A catalytic amount of DMF (2 drops) was added to a stirred suspension of bis-nitroacid (44) (2.00 g, 4.28 mmol) and oxalyl chloride (0.94 mL, 10.70 mmol) in dry THF (20 mL), and the reaction mixture was allowed to stir for 4 h. After evaporation of excess THF in vacuo, the resultant yellow residue was dissolved in dry THF (20 mL) and added dropwise over a period of 25minutes to a vigorously stirred suspension of amine (2) (2.47 g, 10.70 mmol), Et$_3$N (2.50 mL, 17.9 mmol) and ice/water (0.6 mL) cooled in an ice bath. The mixture was then allowed to warm to room temperature for a further 1.5 h. After removal of the THF by evaporation in vacuo, the residue was diluted with water (100 mL) and extracted with ethyl acetate (3 x 100 mL). The combined organic phase was washed with water (3 x 25 mL) and brine (3 x 25 mL), dried (MgSO$_4$), and the solvent removed in vacuo to afford a yellow oil which was purified by flash chromatography (3% MeOH/CHCl$_3$) to afford the bis-amide (49) as a yellow solid (2.05g, 54%). [α]$^{23.8}_D$ = -993° (c 0.033, CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ -0.05 (s, 12H, **H1'**, **H2'**), 0.80 (s, 18H, **H3'**, **H5'**, **H6'**), 1.96-1.99 (m, 2H, **H1**), 2.14-2.16(m, 2H, **H1**), 2.19-2.24 (m, 2H, **H13**), 2.85-2.89 (m, 2H, **H2**) 3.16-3.19 (m, 4H, **H11**), 3.63-3.66 (m, 4H, **H3**), 3.81 (s, 6H, **OMe**), 3.99-4.10 (m, 2H, **H3**), 4.23 (t, 4H, J = 5.3 Hz, **H12**), 4.38 (bs, 2H, **OH**); 5.20-5.25 (m, 2H, **H11a**), 6.65 (s, 2H, **H6**), 7.55 (s, 2H, **H9**). ^{13}C-NMR (CDCl$_3$) : δ -5.35 **(C1', C2')**, 18.2 **(C4')**, 25.8 **(C3', C5', C6')**, 28.9 **(C13)**, 36.1 **(C1)**, 54.9 **(CH$_3$O)**, 56.6 **(C4)**, 57.3 **(C12)**, 65.0 **(C3)**, 70.0 **(C2)**, 108.0 **(C6)**, 109.4 **(C9)**, 128.2 **(Q)**, 137.2 **(Q)**, 148.1 **(Q)**, 148.5 **(Q)**, 154.5 **(Q)**, 166.5 **(Q)**. IR (thin film): ν = 3392 (OH), 2950, 2856, 1623 (C=O), 1577 (C arom), 1524 (NO$_2$), 1459, 1432, 1381, 1338 (C-CH$_3$), 1278 (CH$_3$-Si), 1219 (t-Bu), 1184 (t-Bu), 1075 1053, 1004, 938, 914, 837, 778, 724, 668, 649, cm^{-1}. MS (FAB) m/z (relative intensity) : 894 (M$^+$, 8), 893 (19), 878 (6), 835 (2), 779 (9), 761 (6), 517 (3), 459 (5), 258 (7), 100 (3), 86 (4), 75 (29), 73 (100), 59 (17), 58 (6).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (50)

[0070] A slurry of 10% Pd/C (155 mg) in ethyl acetate (20 mL) was added to a solution of the bis-amide (49) (1.55 g, 1.73 mmol) in ethanol (100 mL). The reaction mixture was hydrogenated (Parr apparatus) for 16 h. The reaction mixture was filtered 'through Celite and the solvent was removed under reduced pressure to give a yellow oil (50) in

quantitative yield (1.44 g, 100%). ^{1}H NMR (CDCl$_3$) : δ 0.00 (s, 12H, **H1', H2'**), 0.88 (s, 18H, **H3', H5', H6'**), 2.00-2.25 (m, 6H, **H1, H13**), 3.50-3.72 (m, 12H, **H2, H3, H11, H11a**), 3.74 (s, 6H, **OMe**), 4.16-4.20 (m, 4H, **H3**), 4.30-4.35 (m, 4H, **H12**), 4.49 (bs, 2H, **OH**); 6.23 (s, 2H, **H9**), 6.64 (s, 2H, **H6**) ^{13}C-NMR (CDCl$_3$) : δ -5.5 (**C1', C2'**), 18.1 (**C4'**), 25.8 (**C3', C5', C6'**), 29.6 (**C13**), 35.2 (**C1**), 56.7 (**CH$_3$O**), 62.2 (**C4**), 64.1 (**C3**), 70.0 (**C2**), 102.2 (**C9**), 112.6 (**C6**), 140.4 (**Q**), 141.1 (**Q**), 150.6 (**Q**), 170.1 (**Q**); IR (neat): ν = 3359 (OH), 2929, 2856, 1621 (C=O), 1591 (C arom), 1469, 1433, 1406, 1358, 1346 (C-CH$_3$), 1261 (CH$_3$-Si), 1232 (*t*-Bu), 1175 (*t*-Bu), 1117, 1056, 1006, 866, 835, 776 cm^{-1}. MS (FAB) m/z (relative intensity) : 834 (M$^{+\cdot}$, 13), 833 (18), 773 (9), 602 (13), 399 (7), 371 (34), 232 (9), 206 (22), 192 (14), 176 (13), 166 (44), 150 (8), 100 (10), 73 (100).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-*N*-allyloxycarbonyl 5-methoxy-1,4-phenyl-ene)-carbonyl]]-bis[(2S, 4R)-2-*t*-butyldimethylsilyloxymethyl-4-hydroxy-pyrrolidine (51)

[0071] A solution of the bis-amide (**50**) (2.76 g, 3.31 mmol) and pyridine (1.10 mL, 13.60 mmol) in dried DCM (100 mL) was cooled to 0°C. Allyl chloroformate (0.80 mL, 7.53 mmol) in DCM (50 mL) was added dropwise and the resulting mixture allowed to warm to room temperature and stirred for 16h. The reaction mixture was diluted with DCM (200 mL) and washed with 1 M CuSO$_4$ (3 x 50 mL), water (1 x 50 mL) and brine (1 x 50 mL) before drying (MgSO$_4$). Evaporation of the solvent under reduced pressure followed by flash column chromatography (2.5% MeOH/DCM) afforded (**51**) as a yellow solid (3.24 g, 97%). [α]$^{20.1}_D$ = -571° (c 0.007, CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ 0.00 (s, 12H, **H1', H2'**), 0.89 (s, 18H, **H3', H5', H6'**), 2.0.3-2.36 (m, 6H, **H1, H13**), 3.51-3.58 (m, 6H, **H2, H3**), 3.77 (s, 6H, **OMe**), 4.20-4.26 (m, 8H, **H11, H12**), 4.28-4.30 (m, 2H, **H11a**), 4.56-4.60 (m, 6H, **H8', OH**), 5.25 (dd, $J_{1,2}$= 1.5 Hz, $J_{1,3}$ = 15.0 Hz, 4H, **H10'**), 5.90-5.95 (m, 2H, **H9'**), 6.73 (s, 2H, **H6**), 7.63 (s, 2H, **H9**), 8.80 (s, 2H, **NH**). ^{13}C NMR (CDCl$_3$): δ -5.42 (**C1', C2'**), 25.8 (**C3', C5', C6'**), 29.2 (**C13**), 35.4 (**C1**), 56.3 (**CH$_3$O**), 57.1 (**C11a**), 59.8 (**C11**), 62.2 (**C3**), 65.1 (**C12**), 65.7 (**C8'**), 70.5 (**C2**), 106.3 (**C9**), 111.5 (**C6**), 116.5 (**Q**), 118.1 (**C10'**), 131.7 (**Q**), 132.5 (**C9'**), 144.3 (**Q**), 150.3 (**Q**), 153.8 (**Q**), 169.5 (**Q**). IR (neat): ν = 3351 (OH), 2931, 2857, 1762 (Alloc C=O), 1722, 1603 (C=O), 1521 (C arom), 1463, 1404, 1264 (CH$_3$-Si), 1222 (*t*-Bu), 1106 (*t*-Bu), 1053, 1015, 936, 872, 837, 775, 629, cm^{-1}.

1,1'-[[(Propane-1,3-diyl) dioxy]bis[2-amino-*N*-allyloxycarbonyl-5-methoxy-1,4-phenylene)-carbonyl]]-bis[(2S)-2-*t*-butyldimethylsilyloxymethyl-4-oxo-pyrrolidine (52)

[0072] A solution of dimethyl sulphoxide (2.10 mL, 28.5 mmol) in dry DCM (20 mL) was added dropwise over a 15minutes period to a stirred, cooled (-45°C) solution of oxalyl chloride (1.27 mL, 14.60 mmol) in DCM (30 mL). After 35minutes, a solution of alcohol (**51**) (2.54g, 2.53 mmol) in DCM (20 mL) was added dropwise over a period of 15minutes to the reaction mixture at -45°C. After 45 minutes a solution of triethylamine (5.75 mL, 40.3 mmol) in DCM (20 mL) was added over a period of 15minutes and the reaction mixture stirred at -45°C for 30minutes before warming to room temperature over 45 minutes. The mixture was then washed with 1 M CuSO$_4$ (3 x 50 mL), water (2 x 50 mL) and brine (1 x 50 mL) before drying (MgSO$_4$) and concentrating in *vacuo* to give (**52**) as a yellow solid (2.46g, 97%). ^{1}H NMR (CDCl$_3$): δ 0.00 (s, 12H, **H1', H2'**), 0.86 (s, 18H, **H3, H5', H6'**), 2.50 -2.63 (m, 6H, **H1, H13**), 3.63-3.70 (m, 4H, **H3**), 3.80 (s, 6H, **OMe**), 3.93-3.97 (m, 6H, **H11, H11a**), 4.29-4.32 (m, 4H, **H12**), 4.62 (d, 4H, *J* = 5.7 Hz, **H8'**), 5.27-5.32 (m, 4H, **H10'**), 5.98-6.03 (m, 2H, **H9'**), 6.74 (s, 2H, **H6**), 7.74 (s, 2H, **H9**), 8.80 (s, 2H, **NH**). ^{13}C NMR (CDCl$_3$): δ -5.76 (**C1', C2'**), 18.0 (**C4'**) 25.7 (**C3', C5', C6'**), 28.8 (**C13**), 39.6 (**C1**), 55.0 (**C3**), 56.4 (**CH$_3$O**), 65.3 (**C12**), 65.8 (**C8'**), 105.9 (**C9**), 110.7 (**C6**), 118.2 (**C10'**), 132.4 (**C9'**), 150.7 (**Q**), 153.5 (**Q**), 169.1 (**Q**), 210.0 (**C2**). IR (neat): ν = 3308 (OH), 2931, 2856, 1765 (Alloc C=O), 1730, 1624 (C=O), 1602 (C=O), 1522 (C arom), 1468, 1407, 1332, 1259 (CH$_3$-Si), 1204 (*t*-Bu), 1105 (*t*-Bu), 1053, 1010, 937, 870, 837, 808, 778, 674, 657 cm^{-1}.

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S)-2-*t*-butyldimethylsilyloxymethyl-4-methylidene-2,3-dihydropyrrole] (206)

[0073] A solution of potassium-*t*-butoxide in dry THF (0.5 M, 4.00 mL, 2.00 mmol) was added to as suspension of methyltriphenylphosphonium bromide (0.716 g, 2.00 mmol) in dry THF (2.00 mL). The resulting yellow ylide suspension was allowed to stir at 0°C for 2 hours before the addition of a solution of the bis-ketone **52** (0.50 g, 0.50 mmol) in THF (10 mL) at 10°C. The reaction mixture was allowed to warm to room temperature and stirring was continued for a further hour.

[0074] The reaction mixture was partitioned between ethyl acetate (15 mL) and water (15 mL) and the organic layer was washed the sat. sodium chloride (20 mL) and dried over magnesium sulphate. Removal of excess solvent gave a brown oil that was subjected to flash column chromatography (50% ethyl acetate, 50% 40-60° petroleum ether) to afford the product as a yellow glass 206 (250 mg, 51%). [α]$^{23.4}_D$ = -32 ° (c 0.265, CHCl$_3$). ^{1}H NMR (CDCl$_3$): δ 0.00 (s, 12H), 0.88 (s, 18H), 2.37-2.40 (m, 2H), 2.69-2.75 (m, 4H), 3.80-4.62 (m, 20H), 4.61-4.63 (m, 4H), 4.98 (bs, 4H), 5.30-5.38 (m, 4H), 5.94-6.00 (m, 2H), 6.81 (s, 2H), 7.84 (s, 2H), 8.80 (bs, 2H).

1,1'-[[(Propane-1,3-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S)-2-hydroxymethyl-4-methylidene-2,3-dihydropyrrole] (78)

[0075]   An aliquot of hydrogen fluoride/pyridine complex (0.8 mL, 70% HF, 30 % pyridine) was added to a solution of the bis-silyl ether 206 (285 mg, 0.287 mmol) in THF (10 mL) at 0°C under a nitrogen atmosphere. Stirring was continued at 0°C for 30 minutes and the reaction mixture was then allowed to rise to room temperature over a 1 hour period. The reaction mixture was neutralised with sodium bicarbonate and extracted with dichloromethane (3 x 30 mL). The combined organic phase was washed with brine and dried over magnesium sulphate. Removal of excess solvent under reduced pressure afforded the product 78 as a yellow gum (218 mg).

1,1'[[(Propane-1,3-diyl)dioxy]bis(11S,11aS)-10-(allyloxycarbonyl)-11-hydroxy-7-methoxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4-benzodiazepin-5-one] (79)

[0076]   A solution of dimethyl sulphoxide (0.55 mL, 7.75 mmol) in dry dichloromethane (10 mL) was added dropwise, over a 15 minute period, to a stirred solution of oxalyl chloride (0.32 mL, 3.67 mmol) in dichloromethane (10 mL) at -45°C under a nitrogen atmosphere. The reaction mixture was allowed to stir for 35 minutes at -45°C followed by addition of the diol 78 (1.01 g, 1.32 mmol) in dichloromethane (10 mL), at the same temperature, over 15 minutes. After a further 45 minutes a solution of triethylamine (1.50 mL, 10.76 mmol) in dichloromethane (10 mL) was added over a period of 15 minutes.

[0077]   The reaction mixture was allowed to stir at -45°C for 30 minutes before being allowed to warm to room temperature over 45 minutes. The reaction mixture was diluted with water and the phases were allowed to separate. The organic phase was washed with 1M HCl (3 x 50 mL), sat. sodium chloride (50 mL) and dried over magnesium sulphate. Removal of excess solvent yielded the crude product, which was purified by flash column chromatography (1.5% methanol, 98.5% chloroform) to afford the product 79 (0.785 g, 77%).

1,1'[[(propane-1,3-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4] benzodiazein-5-one] (80, SJG-136)

[0078]   A catalytic amount of tetrakis(triphenylphosphine)palladium (21 mg, 0.018 mmol) was added to a stirred solution of the bis-alloc-carbinolamine 79 (250 mg, 0.33 mmol), triphenylphosphine (10 mg, 0.033 mmol) and pyrrolidine (0.05 mL, 0.66 mmol) in dry CH_2Cl_2 (30 mL) at 0°C (ice/acetone) under a nitrogen. atmosphere. The reaction mixture was allowed to stir for 2 hours before warming to room temperature over 1 hour.. The solvent was evaporated under reduced pressure and the resulting residue subjected to flash chromatography (98% $CHCl_3$/MeOH) to give the bis-imine target molecule 80 (SJG-136). The reaction mixture was allowed to stir for 1 hour at 0°C, the ice bath was removed and the reaction mixture was allowed to warm to room temperature a mild exotherm, c. 40°C, accompanied by the evolution of NO_2 occurred at this stage. After the exotherm had subsided stirring at room temperature was continued for 2 hours. The reaction mixture was poured into ice water and the aqueous suspension allowed to stir for 1 h. The resulting yellow precipitate was collected by vacuum filtration and dried in air to afford the desired bis nitro compound (209) (14.23 g, 98 %): ^{1}H NMR (400 MHz, $CDCl_3$ + DMSO) δ 7.45 (s, 2H), 7.09 (s, 2H), 4.14 (t, 4H, J = 6.31 Hz), 3.97 (s, 6H), 3.90 (s, 6H), 2.20-1.94 (m, 4H), 1.75-1.70 (m, 2H).

1',5'-Bis(4-carboxy-2-methoxy-5-nitrophenoxy) pentane (210)

[0079]   A suspension of the ester 209 (9.0 g, 17.2 mmol) in aqueous sodium hydroxide (1 M, 180 mL) and THF (180 mL) was allowed to stir until a homogenous solution was obtained (2 days). THF was evaporated under reduced pressure and the resulting aqueous suspension was filtered to remove any unreacted starting material. The filtrate was adjusted to pH 1, the precipitated product was collected by filtration and air dried to afford the desired bis-acid (210) (8.88 g). A higher than theoretical yield was obtained due to the inclusion of the sodium salt of acid. The salt may be removed by dissolving the bulk of the material in THF and removing the insoluble material by filtration: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 (s, 2H), 7.16 (s, 2H), 4.12 (t, 4H, J = 6.59 Hz), 3.95 (s, 6H), 2.00-1.85 (m, 4H), 1.75-1.67 (m, 2H).

Assembling the Bis Ketone Intermediate

1,1'-[[(Pentane-1,5-diyl)dioxy]bis[2-nitro-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine] (211)

[0080]   A catalytic amount of DMF (5 drops) was added to as stirred suspension of the acid 210 (5.39 g, 10.9 mmol) and oxalyl

[0081] A catalytic amount *of* DMF (5 *drops*) was added to as stirred suspension of the acid **210** (5.39 g, 10.9 mmol) and oxalyl chloride (3.47 g, 2.38 mL, 21.3 mmol) in anhydrous THF (50 *mL*). Initial effervescence *was* observed followed by the formation of a homogenous solution, however after stirring overnight a suspension of the newly formed acid chloride was formed. Excess THF and oxalyl chloride was removed by rotary evaporation under reduced pressure and the acid chloride was resuspended in fresh THF (49 mL). The acid chloride solution was added dropwise to a solution of the (2S, 4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine (2) (6.3 g, 27.3 mmol), triethylamine (4.42 g, 6.09 mL, 43.7 mmol) and water (1.47 mL) in THF (33 mL) at 0°C under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and stirring was continued for 3 h. Excess THF was removed by rotary evaporation under reduced pressure and the resulting residue was partitioned between water (300 mL) and ethyl acetate (300 mL). The layers were allowed to separate and the aqueous layer was extracted with ethyl acetate (3 x 150 mL). The combined organic layers were then washed with ammonium chloride (150 mL), sat. sodium bicarbonate (150 mL), brine (150 mL) and dried over magnesium sulphate. Filtration followed by rotary evaporation under reduced pressure afforded the crude product as a dark oil. The crude product was subjected to flash column chromatography (3% methanol, 97% chloroform) and removal of excess eluent isolated (**211**) (3.70 g, 37% yield): ^{1}H NMR (270 MHz, CDCl$_3$) δ 7.65 (s, 2H), 6.77 (s, 2H), 4.52 (bs, 2H), 4.40 (bs, 2H), 4.17-4.10 (m, 6H), 3.92 (s, 6H), 3.77 (d, 2H, J = 10.26 Hz), 3.32 (td, 2H, J = 4.40, 11.35 Hz), 3.08 (d, 2H, J = 11.35 Hz), 2.37-2.27 (m, 2H), 2.10-2.00 (m, 6H), 1.75-1.60 (m, 2H), 0.91 (s, 18H), 0.10 (s, 12H).

1,1'-[[(Pentane-1,5-diyl)dioxy]bis[2-amino-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S,4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine] (212)

[0082] A methanolic solution of hydrazine hydrate (1.25 mL, 1.29 g, 40.2 mmol of hydrazine, 20 mL of methanol) was added dropwise to a solution of the bis-nitro compound **211** (3.6 g, 3.91 mmol) in methanol (68 mL) gently refluxing over Raney nickel (510 mg of a thick slurry). After 5 minutes at reflux TLC (10% MeOH, 90% chloroform) revealed the incomplete consumption of starting material. The reaction mixture was treated with additional Raney nickel (c 510 mg) and hydrazine (1.25 mL) in methanol (20 mL) resulting in complete consumption of starting material. Excess Raney nickel was added to the reaction mixture to decompose unreacted hydrazine hydrate and the reaction mixture was then allowed to cool. The reaction mixture was filtered through celite to remove excess Raney nickel and the filter pad washed with additional methanol (Caution! Raney nickel is pyrophoric, do not allow filter pad to dry, use conc. HCl to destroy nickel). The combined filtrate was evaporated by rotary evaporation under reduced pressure and the residue re-dissolved in dichloromethane. The dichloromethane solution was dried over magnesium sulphate (to remove water associated with the hydrazine), filtered and evaporated to afford the product (**212**) as a foam (3.37 g, 91%): ^{1}H NMR (270 MHz, CDCl$_3$) δ 6.69 (s, 2H), 6.24 (s, 2H), 4.40-3.40 (m, 28H), 2.40-1.60 (m, 10H), 0.88 (s, 18H), 0.03 (s, 12H).

1,1,-[[(Pentane-1,5-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl]]-bis [(2S, 4R)-2-t-butyldimethylsilyloxymethyl-4-hydroxypyrrolidine] (213)

[0083] A solution of allyl chloroformate (0.806 mL, 0.916 g, 7.6 mmol) in dry dichloromethane (63 mL) was added, dropwise, to a solution of the bis-amine **212** (3.27 g, 3.8 mmol) and pyridine (1.26 g, 1.29 mL, 15.9 mmol) in dichloromethane (128 mL) at 0°C under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and to stir for 16 h. At which time TLC (10% MeOH, 90% Chloroform) revealed reaction to be complete. The reaction mixture was diluted with dichloromethane (40 mL) and washed with sat. copper II sulphate (2 x 140 mL), water (120 mL) and sat. sodium chloride (120 mL). The organic phase was dried over magnesium sulphate, filtered and evaporated under reduced pressure to afford 213 as a foam (3.60 g, 92%): ^{1}H NMR (270 MHz, CDCl$_3$) δ 8.87 (bs, 2H), 7.66 (s, 2H), 6.77 (s, 2H), 6.05-5.80 (m, 2H), 5.40-5.15 (m, 4H), 4.70-4.50 (m, 6H), 4.38 (bs, 2H), 4.20-4.00 (m, 4H), 3.78 (s, 6H), 3.70-3.40 (m, 8H), 2.40-2.20 (m, 2H), 2.10-1.80 (m, 6H), 1.75-1.55 (m, 2H), 0.89 (s, 18H), 0.04 (s, 12H).

1,1'-[[(Pentane-1,5-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S)-2-t-butyldimethylsilyloxymethyl-4-oxo-pyrrolidine] (214)

[0084] A solution of dimethyl sulphoxide (1.47 mL, 1.62 g, 20.7 mmol) in dry dichloromethane (32 mL) was added dropwise over 45 minutes to a stirred solution of oxalyl chloride (5.18 mL of a 2 M solution in dichloromethane, 10.35 mmol) at - 60°C under a nitrogen atmosphere. After stirring at - 50°C for 30 minutes, a solution of the bis-alcohol **213** (3.55 g, 3.45 mmol) in dichloromethane (53 mL) was added dropwise over a period of 50 minutes. The reaction mixture was allowed to stir at -60°C for 30 minutes prior to the dropwise addition of a solution of triethylamine (4.75 g, 6.54 mL, 46.9 mmol) in dichloromethane (27 mL). Stirring was continued at - 60°C for 45 minutes and then allowed to warm to 0°C. The reaction mixture was diluted with dichloromethane (20 mL), washed with cold 1 M HCl (2 x 100 mL), sat. sodium chloride (100 mL) and dried over magnesium sulphate. Removal of excess solvent afforded the crude bis-

ketone which was purified by flash column chromatography (50% ethyl acetate, 50% 40-60° petroleum ether) to yield the pure bis-ketone (214) as a pale yellow foam (2.54 g, 72%): ^{1}H NMR (270 MHz, CDCl$_3$) δ 8.69 (bs, 2H), 7.78 (s, 2H), 6.75 (s, 2H), 6.05-5.80 (m, 2H), 5.40-5.20 (m, 4H), 4.65-4.60 (m, 4H), 4.20-3.60 (m, 20H), 2.74 (dd, 2H, J = 9.25, 18.1 Hz), 2.51 (d, 2H, J = 17.4 Hz), 2.00-1.90 (m, 4H), 1.75-1.65 (m, 2H), 0.87 (s, 18H), 0.05 (s, 12H).

Elaboration of bis Ketone and Preparation of the Target Molecule

1,1'-[[(Pentane-1,5-diyl)dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl])]-bis[(2S)-2-t-butyldimethylsilyloxymethyl-4-methylidene-2,3-dihydropyrrole] (215)

[0085] A solution of potassium-t-butoxide in dry THF (0.5 M, 25.2 mL, 12.6 mmol) was added dropwise to a suspension of methyltriphenylphosphonium bromide (4.50 g, 12.6 mmol) in dry THF (15 mL). The resulting yellow ylide suspension was allowed to stir at 0°C for 2 hours before the addition of a solution of the bis-ketone 214 (2.48 g, 2.42 mmol) in THF (10 mL) at 10°C. The reaction mixture was allowed to warm to room temperature and stirring was continued for a further hour. The reaction mixture was partitioned between ethyl acetate (100 mL) and water (100 mL) and the organic layer was washed with sat. sodium chloride (200 mL) and dried over magnesium sulphate. Removal of excess solvent gave a brown oil that was subjected to flash column chromatography (50% ethyl acetate, 50% 40-60° petroleum ether) to afford the product (215) as a yellow glass (865 mg, 35%): ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.90 (bs, 2H), 7.83 (s, 2H), 6.82 (s, 2H), 6.05-5.90 (m, 2H), 5.40-5.20 (m, 4H), 4.99 (bs, 2H), 4.91 (bs, 2H), 4.65-4.60 (m, 4H), 4.20-3.60 (m, 20H), 2.70 (bs, 4H), 2.00-1.90 (m, 4H), 1.75-1.63 (m, 2H), 0.88 (s, 18H), 0.03 (s, 12H).

1,1'-[[(Pentane-1,5-diyl) dioxy]bis[2-amino-N-allyloxycarbonyl-5-methoxy-1,4-phenylene)carbonyl]]-bis[(2S)-2-hydroxymethyl-4-methylidene-2,3-dihydropyrrole] (216)

[0086] A solution of TBAF (3.02 mL of a 1 M solution in THF, 3.02 mmol) was added to the bis-silyl ether (215) (1.23 g, 1.21 mmol) in THF (30 mL) at 0°C (ice/acetone). The reaction mixture was allowed to warm to room temperature and to stir overnight, the following day, TLC (50:50 EtOAc/Pet-Ether 40°-60°) revealed the complete disappearance of starting material. Saturated NH$_4$Cl (150 mL) was added and the reaction mixture extracted with EtOAc (3 X 60 mL), washed with sat. sodium chloride (150 mL), dried (MgSO$_4$), filtered and evaporated in vacuo to give a yellow oil. Purification by flash chromatography (97% CHCl$_3$/ 3%MeOH) provided the pure alcohol (216) (916 mg, 96%): ^{1}H NMR (400 MHz, CDCl$_3$) δ 8.61 (bs, 2H), 7.58 (s, 2H), 6.79 (s, 2H), 6.05-5.90 (m, 2H), 5.40-5.20 (m, 4H), 5.01 (bs, 2H), 4.93 (bs, 2H), 4.65-4.60 (m, 4H), 4.20-3.60 (m, 20H), 2.76 (dd, 2H, , J = 8.42, 15.74 Hz), 2.47 (d, 2H, J = 15.93 Hz), 2.00-1.90 (m, 4H), 1.80-1.63 (m, 2H).

1,1'[[(Pentane-1,5-diyl)dioxy]bis(11S,11aS)-10-(allyloxycarbonyl)-11-hydroxy-7-methoxy-2-methylidene-1,2,3,10,11,11a-hexahydro-5H-pyrrolo[2,1-c][1,4-benzodiazepin-5-one] (217)

[0087] A solution of dimethyl sulphoxide (0.57 mL, 0.63 g, 8.07 mmol) in dry dichloromethane (17 mL) was added dropwise, over a 40 minute period, to a stirred solution of oxalyl chloride (2.02 mL, of a 2 M solution, 4.04 mmol) at -45°C under a nitrogen atmosphere. The reaction mixture was allowed to stir for 40 minutes at - 45°C followed by addition of the diol 216 (0.89 g, 1.12 mmol) in dichloromethane (17 mL), at the same temperature, over 15 minutes. After a further 60 minutes a solution of triethylamine (1.31 mL, 9.42 mmol) in dichloromethane (9 mL) was added over a period of 40 minutes. The reaction mixture was allowed to stir at - 45°C for 40 minutes before being allowed to warm to room temperature over 45 minutes. The reaction mixture was diluted with water and the phases were allowed to separate. The organic phase was washed with 1 M HCl (2 x 40 mL), water (40 mL), sat. sodium chloride (40 mL) and dried over magnesium sulphate. Removal of excess solvent yielded the crude product, which was purified by flash column chromatography (1% methanol, 99% chloroform) to afford the product 217 (0.175 g, 20%): ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.22 (s, 2H), 6.65 (s, 2H), 5.82-5.70 (m, 2H), 5.58 (d, 2H, J = 9.70 Hz), 5.25-5.00 (m, 8H), 5.75-4.35 (m, 4H), 4.30 (d, 2H, J = 16.10 Hz), 4.15 (d, 2H, J = 17.03 Hz), 4.01 (t, 4H, J = 6.32 Hz), 3.90 (s, 6H), 3.64 (t, 2H, J = 8.70 Hz), 3.00-2.85 (m, 2H), 2.71 (d, 2H, J = 16.29 Hz), 2.00-1.85 (m, 4H), 1.70-1.60 (m, 2H).

1,1'[[(pentane-1,5-diyl)dioxy]bis[(11aS)-7-methoxy-2-methylidene-1,2,3,11a-tetrahydro-5H-pyrrolo[2,1-c][1,4] benzodiazepin-5-one] (218)

[0088] A catalytic amount of tetrakis(triphenylphosphine)palladium (13 mg, 11.2 mmol) was added to a stirred solution of the bis-alloc-carbinolamine (217) (170 mg, 0.22 mmol), triphenylphosphine (5.7 mg, 21.6 mmol) and pyrrolidine (31 mg, 37.3 mL 0.45 mmol) in DCM (13 mL) at 0°C (ice/acetone) under a nitrogen atmosphere. The reaction mixture was allowed to warm to room temperature and the progress of reaction monitored by TLC (95% CHCl$_3$/MeOH). After 2

hours TLC revealed the reaction was complete to give a spot, which fluoresced brightly under UV light. The solvent was evaporated under reduced pressure and the resulting residue subjected to flash chromatography (99% to 98 $CHCl_3$/MeOH) to give the bis-imine target molecule **218** as a pale yellow glass (84.5 mg, 75%) which was repeatedly evaporated in *vacuo* with $CHCl_3$ to provide the imine form: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, 2H, J = 4.39 Hz), 7.49 (s, 2H), 6.80 (s, 2H), 5.19 (bs, 2H), 5.16 (bs, 2H), 4.28 (bs, 4H), 4.15-4.00 (m, 4H), 3.92 (s, 6H), 3.90-3.80 (m, 2H), 3.12 (dd, 2H, , J = 8.97, 15.93 Hz), 2.95 (d, 2H, J = 15.93 Hz), 2.00-1.85 (m, 4H), 1.72-1.67 (m, 2H).

Cytotoxicity Data

NCI *In Vitro* Cytotoxicity Studies

[0089] The National Cancer Institute (NCI), Bethesda, Maryland, USA has available an in *vitro* cytotoxicity screen which consists of approximately 60 human tumour cell lines against which compounds are tested at a minimum of five concentrations each differing 10-fold. A 48 hour continuous exposure protocol is used, where cell viability or growth is estimated with an SRB protein assay.

Method

[0090] The test compounds were evaluated against approximately 60 human tumour cell lines. The NCI screening procedures were described in detail by Monks and co-workers (Monks, A *et al.*, Journal of the National Cancer Institute, 1991, 83, 757). Briefly, cell suspensions were diluted according to the particular cell type and the expected target cell density (5000-40,000 cells per well based on cell growth characteristics), and added by pipette (100 μL) into 96-well microtitre plates. The cells were allowed a preincubation period of 24 hours at 37°C for stabilisation. Dilutions at twice the intended test concentration were added at time zero in 100 μL aliquots to the wells. The test compounds were evaluated at five 10-fold dilutions (10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} μM). The test compounds were incubated for 48 hours in 5% CO_2 atmosphere and 100% humidity. The cells were then assayed using the sulphorhodamine B assay. A plate reader was used to read the optical densities and a microcomputer processed the readings into LC_{50} values, which is the dosage required to kill half of the cells.

[0091] The results presented in examples 2 to 4 are LC_{50} values which are below 10μM, which is taken to be the dividing line between cytotoxicity and non-cytotoxicity.

NCI Hollow Fibre Assay for Preliminary *In Vivo* Testing

[0092] The Biological testing Branch of the Developmental Therapeutics Program of the NCI has adopted a preliminary in *vivo* screening tool for assessing the potential anticancer activity of compounds identified by the large scale *in vitro* cell screen. For these assays, human tumour cells are cultivated in polyvinylidene (PVDF) hollow fibres, and a sample of each cell line is implanted into each of two physiologic compartments (intraperitoneal and subcutaneaous) in mice. Each test mouse received a total of 6 fibres (3 intraperitoneally and 3 subcutaneously) representing 3 distinct cancer cell lines. These mice are treated with potential antitumour compounds at each of 2 test doses by the intraperitoneal route using a QD x 4 treatment schedule. Vehicle controls consist of 6 mice receiving the compound diluent only. The fibre cultures are collected on the day following the last day of treatment. To assess anticancer effects, the viable cell mass is determined for each of the cell lines using a formazyn dye (MTT) conversion assay. From this, the %T/C can be calculated using the average optical density of compound treated samples divided by the average optical; density of the vehicle controls. In addition, the net increase in cell mass can be determined for each sample, as a sample of fibre cultures are assessed for viable cell mass on the day of implantation into mice. Thus, the cytostatic and cytocidal capacities of the test compound can be assessed.

[0093] Generally, each compound is tested against a minimum of 12 human cancer cell lines. This represents a total of 4 experiments since each experiment contains 3 cell lines. The data are reported as %T/C for each of the 2 compound doses against each of the cell lines with separate values calculated for the intraperitoneal and subcutaneous samples.

[0094] Compounds are selected for further in *vivo* testing in standard subcutaneous xenograft models on the basis of several hollow fibre assay criteria. These include: (1) a %T/C of 50 or less in 10 of the 48 possible test combinations (12 cell lines X 2 sites X 2 compound doses); (2) activity at a distance (intraperitoneal drug/subcutaneous culture) in a minimum of 4 of the 24 possible combinations; and/or (3) a net cell kill of 1 or more of the cell lines in either implant site. To simplify evaluation, a points system has been adopted which allows rapid evaluation of the activity of a given compound. For this, a value of 2 is assigned for each compound dose which results in a 50% or greater reduction in viable cell mass. The intraperitoneal and subcutaneous samples are scored separately so that criteria (1) and (2) can be evaluated. Compounds with a combined IP + SC score of 20, a SC score of 8 or a net cell kill of one or more cell lines are referred for xenograft testing. This comparison indicated that there was a very low probability of missing an

active compound if the hollow fibre assay was used as the initial *in vivo* screening tool. In addition to these criteria, other factors (e.g. unique structure, mechanism of action) may result in referral of a compound for xenograft testing without the compound meeting these criteria.

NCI Human Xenograft Studies

[0095] These are carried out on nucle athymic mice with a disabled immune system. The human tumour tissue to be tested is implanted in their flanks, and whilst the control mouse receives no treatment, the others are subjected to varying doses of the test compound, which is administered intraperitoneally. The results are expressed as the toxicity of the compound, the amount of tumour growth, and the inhibition of growth.

Example 2(a) : *In Vitro* Cytotoxicity

[0096] Compound 80 was subjected to the NCI *In Vitro* Cytotoxicity study. The results ($LC_{50};\mu M$) are set out below:

TUMOUR TYPE	CELL-LINE DESIGNATION	UP2001 (80)
		LC_{50} (μM)
Lung	NCI-H460	2.7
Colon	HCC-2998	0.099
CNS	SNB-75	7.5
Melanoma	MALME-3M	0.073
	UACC-62	0.077

[0097] The PBD dimer UP2001 (**80**) exhibited potent and selective cytoxicity activity against the lung cancer cell line NCI-H460, the colon cell line HCC-2998, the CNS cancer cell line SNB-75 and the melanoma cell lines MALME-3M (very potent, 0.08 μM) and UACC-62 (very potent, 0.07 μM), which may be attributable to its ability to cross link DNA.

Example 2(b) : Hollow Fibre Assay

[0098] Compounds 80 underwent the NCI Hollow Fibre Assay, and the results are presented below.

	UP2001 (80)
IP score	40
SC score	14
Total score	54
Cell Kill	Y

[0099] UP2001 has been selected for xenograft studies based on its combined IP + SC score (54) which was greatly in excess of 20, and its SC score which was higher than 8.

Example 2(c) : Human Xenograft Studies

[0100] Human tumour xenograft studies on UP2001 were performed by the Biological Testing Branch of the NCI as described above.
[0101] Athymic nude mice bearing MDA-MB-435 xenografts (human mammary tumour), Ovcar-3 (human ovarian tumour), UACC-62 (human melanoma) or OVCAR-5 (human ovarian tumour) were treated at doses of 0.67 (high), 0.45 (middle) and 0.3 (low) mg/kg/injection given once every 4th day for a total of 3 doses (6 mice per dose level with

20 controls).

[0102] UP2001 (80) was evaluated by measuring the toxicity of the drug and its ability to retard tumour growth.

Tumour	Toxicity			%T/C			% Growth Delay		
	High	Mid	Low	High	Mid	Low	High	Mid	Low
MDA-MB-435	3/6	1/6	2/6	toxic	3	3	41	41	41
OVCAR-3	0/6	0/6	0/6	7	20	46	73	73	9
UACC-62	0/6	0/6	0/6	22	28	67	43	43	43
OVCAR-5	0/6	0/6	0/6	52	45	38	16	28	32

[0103] Toxicity represents the number of mice which died as a result of treatment. %T/C represents the width of the tumours in the "test" mice (T) (as measured with calipers) compared to control untreated mice (C) and presented as a percentage. % Growth Delay represents the increase in the amount of time taken for the tumors to reach an arbitrary size of 250 mg.

[0104] In the MDA-MB-435 xenografts UP2001 restricted tumour growth in treated mice to only 3% of the tumour growth observed in the control population. In addition, a 41% delay in the time taken to reach tumour mass of 250 mg was also observed. Some toxicity towards the hosts was observed even at low dose. A good dose response was observed for UP2001 (80) in the Ovcar-3 xenografts. At the high dose, tumour growth in treated subjects was only 7% of that observed in the control population. At the medium dose the value was 20% and at the low dose the tumours in the treated mice were 46% of the size of the control tumours. At the high dose a 73% growth delay in reaching a tumour mass of 250 mg was observed. No mice died as a result of exposure to UP2001 (80).

[0105] A similar dose response for tumour growth was observed in the UACC-62 xenografts for UP2001 (80). At the high dose treated tumours were 22% of the size of the control tumours. At the medium dose treated tumours were 28% of the size of the control tumours and at the low dose treated tumours were 67% of the size of the control tumours. Again no mice died as a result of exposure to UP2001 (80).

[0106] Results for the human ovarian tumour OVCAR-5 were less clear cut; approximately 50% tumour size reduction was observed and some growth delay was observed but activity appeared to be higher at lower concentrations. However, again, mice died as a result of exposure to UP2001 (80).

[0107] UP2001 (80) was also evaluated against the human CNS tumour SF-295. Athymic nude mice bearing SF-295 were treated at doses of 0.40, 0.27 and 0.18 mg/Kg by injection given intravenously once daily for a total of 5 doses.

Toxicity			%T/C			Tumour Free		
High	Med	Low	High	Med	Low	High	Med	Low
2/6	1/6	2/6	0%	0%	0%	4/4	5/5	3/4

[0108] UP2001 (80) displayed curative properties against SF-295 xenografts. At high and medium doses all the surviving mice were tumour free on day 27 of the experiment. At the lower dose 3 out of 4 mice were tumour free on day 27. Some toxicity was associated with the treatment, 2 mice dying at the high dose, 1 at the medium dose and two at the low dose. The higher intensities of the injection schedule may be reflected in the higher mortality observed.

Example 3: Further results for PBD dimer SJG-136 (UP2001, 80)

[0109] Compound 80 underwent some further assays.

[0110] The first assay, which is described in G.B.Jones, et al., Anti-Cancer Drug Des., 1990, 5, 249, which is incorporated herein by reference, determines the effect of the test compound on the helix melting temperature of DNA. This assay is designed to give an indication of the strength and extent of cross-linking of the DNA strands by the test compound (i.e. a measure of the stabilisation of the DNA upon ligand binding).

[0111] The melting temperature was determined for a 1:5 molar ratio of [ligand][DNA], where the calf thymus DNA concentration is 100 mM in aqueous sodium phosphate buffer (10 mM sodium phosphate + 1 mM EDTA, pH 7.00±0.01). For calf thymus DNA at pH 7.00±0.01, the melting temperature is 67.83±0.06°C (mean value from 30 separate determinations).

[0112] For a 1:5 molar ratio of [PBD]:[DNA], the PBD dimer **80** elevates the helix melting temperature (ΔT_m) of calf thymus DNA by an unprecedented 33.6°C after incubation for 18 hours at 37°C. Under identical conditions, the C-ring-unsubstituted dimer DSB-120:

DSB-120
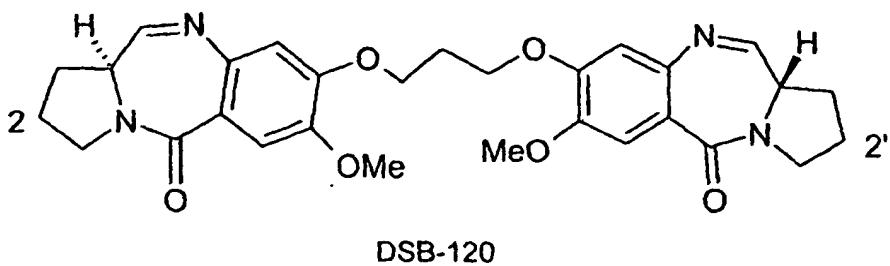
provides a ΔT_m of 15.1°C, demonstrating the extraordinary effect of introducing C2/C2'-unsaturation. In common with other PBD dimers, **80** exerts most of its effect upon the GC-rich or high temperature regions of the DNA melting curves. In a similar fashion to DSB-120, it provides some 60-80% of its stabilising effect without prior incubation, suggesting a kinetic effect in the PBD reactivity profile. However, the comparative ΔT_m curves show that, on a concentration basis alone, SJG-136 is ≥ 10-fold more effective than DSB-120. Even at a [PBD]:[DNA] molar ratio of 1:100, SJG-136 still exhibits significantly better DNA binding affinity than the monomer tomaymycin at a 1:5 [PBD][DNA] molar ratio.
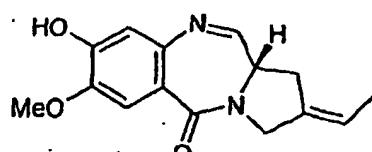
Tomamycin

[0113] The results for a [PBD]:[DNA] ratio of 1:5 are summarised in the table below (All ΔTm values ± 0.1-0.2°C)

Compound	Induced ΔT_m (°C) after incubation at 37°C for		
	0 h	4 h	18 h
SJG-136 (80)	25.7	31.9	33.6
DSB-120	10.2	13.1	15.1
Tomamycin	0.97	2.38	2.56

[0114] The data presented in the above table show that SJG-136 (**80**) is the most potent DNA-stabilising agent known to date according to this particular assay.

[0115] The second assay determined the cytotoxicity of SJG-136 (**80**) in the human ovarian carcinoma cell line A2780 and its cisplatin-resistant subline A2780cisR, and compared this data with the cytotoxicity of the related dimer DSB-120 (see above) and Cisplatin. Relative to the parental line, the A2780cisR subline is known to have elevated GSH levels, an increased level of repair of DNA-cisplatin adducts, and a decreased ability to uptake cisplatin (M.Smellie, *et al., Br. J. Cancer*, 1994, **70**, 48).

[0116] The results, which were obtained by incubating the cells with the compounds for 96 hours at 37°C, and assessing the cell number using Sulforhodamine B, are presented in the table below:

	IC_{50}[a] (µM) for		
	A2780	A2780cis[R]	RF[b]
SJG-136 (80)	0.000023	0.000024	1.1
DSB-120	0.0072	0.21	29.2
Cisplatin	0.265	8.4	32

a Dose of compounds required to inhibit cell growth by 50% compared with control

b RF is the resistance factor (IC_{50} resistant/parent)

[0117] The IC_{50} value for **80** in the A2780 cell line is only 23 pM, representing a 320-fold increase in cytotoxicity compared to DSB-120 (IC_{50} = 7.2 nM). More interestingly, whereas DSB-120 has a reduced potency in the cisplatin-resistant A2780cisR (IC_{50} = 0.21 mM), SJG-136 is almost 9,000-fold more potent in this cell line with a similar IC_{50} value (24 pM) to that in the normal A2780, giving a Resistance Factor of 1.1. The fact that both DSB-120 and cisplatin give Resistance Factors of 29.2 and 32, respectively, across this pair of cell lines suggests that SJG-136 may have potential in the treatment of cisplatin-refractory disease.

Example 4: Ovarian Carcinoma Cytotoxicity Assay

[0118] Compound 80 (and Anthramycin as a comparison) were evaluated for their cytotoxic activity in ovarian cell lines by Dr Lloyd R. Kelland's group at The Institute of Cancer Research, Sutton, UK. The five cell lines investigated were SKOV-3, A2780/A2780cisR and CH1/CH1cisR (cisR denotes that the cell line is resistant to cisplatin).

[0119] Single viable cells were seeded in growth medium (160 µL) in 96-well microtitre plates and allowed to attach overnight. The PBDs were then dissolved in DMSO (to give 20 mM drug concentrations) immediately prior to adding to the cells in quadruplicate wells. The final drug concentrations in the wells ranged from 100 µM to 2.5 nM as follows: 100, 25, 10, 2.5, 1 µM, 250, 100, 25, 10, 2.5 nM (drugs were diluted in growth medium and then 40 µL added to the existing well volume of 160 µL to give final concentrations as above). After 96 hours, the medium was removed and the remaining cells fixed by exposure to 10% trichloroacetic acid on ice for 30 minutes. The wells were then washed 3-4 times with tap water, air dried overnight and treated with 100 µL of sulphorhodamine B (0.4%) dissolved in 1% acetic acid. Staining was allowed to continue for 10-15 minutes, then the wells were washed 3-4 times with 1% acetic acid, air dried and then added to Tris base (100 µL of 10 mM). Plates were then shaken and absorbance readings at 540 nm were determined using a plate reader. By using the Quattro-Pro software package, the IC_{50} values were calculated from plots of concentration versus percentage absorbance (compared with 8 untreated wells).

	$IC_{50}/\mu M$				
UP No.	A2780	A2780cis	CH1	CH1cisR	Skov3
Anthramycin	0.155	0.16	0.062	0.05	0.16
UP2001 (80)	0.000023	0.000024	0.00012	0.0006	0.0091

[0120] UP2001 (**80**) exhibits cytotoxicity at picomolar/sub nanomolar levels across the ovarian cell line panel. The potency of the molecule is probably due to its cross-linking properties coupled with the effect of exo saturation.

Claims

1. A compound of the formula:
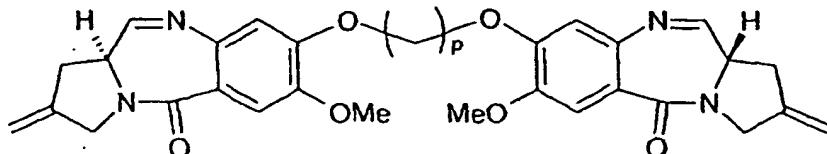
wherein p is 3.

2. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier or diluent.

3. A compound according to claim 1 for use in a method of treatment by therapy.

4. The use of a compound according to claim 1 to prepare a medicament for the treatment of a proliferative disease.

5. The use of a compound according to claim 1 to prepare a medicament for the treatment of a viral, parasitic or bacterial infection.

6. A process for preparing a compound according to claim 1.

7. The use of a compound according to claim 1 for the preparation of a medicament for the treatment of cisplatin-refactory disease.

Patentansprüche

1. Verbindung der Formel:
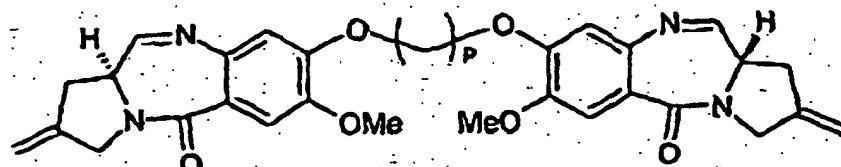
worin p = 3 ist.

2. Pharmazeutische Zusammensetzung, die eine Verbindung nach Anspruch 1 und einen pharmazeutisch annehm-baren Träger oder Verdünner umfasst.

3. Verbindung nach Anspruch 1 zur Verweidung bei der Behandlungsmethode durch Therapie.

4. Verwendung einer Verbindung nach Anspruch 1 zur Herstellung eines Medikaments zur Behandlung einer proli-ferativen Erkrankung.

5. Verwendung einer Verbindung nach Anspruch 1 zur Herstellung eines Medikaments zur Behandlung einer viralen, parasitären oder bakteriellen Infektion.

6. Verfahren zur Herstellung einer Verbindung nach Anspruch 1.

7. Verwendung einer Verbindung nach Anspruch 1 zur Herstellung eines Medikaments zur Behandlung von Cisplatin-Refactory-Erkrankung.

Revendications

1. Composé de la formule:
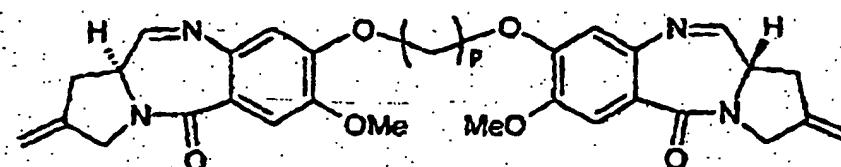
où p est 3.

2. Composition pharmaceutique comprenant un composé selon la revendication 1 et un support ou diluant pharmaceutiquement acceptable.

3. Composé selon la revendication 1 pour une utilisation dans une méthode pour un traitement par thérapie.

4. Utilisation d'un composé selon la revendication 1 pour préparer un médicament pour le traitement d'une maladie proliférative.

5. Utilisation d'un composé selon les revendication 1 pour préparer un médicament pour le traitement d'une infection virale, parasitaire ou bactérienne.

6. Procédé pour la préparation d'un composé selon la revendication 1.

7. Utilisation d'un composé selon la revendication 1 pour la préparation d'un médicament pour le traitement d'une maladie réfractaire au cisplatine.
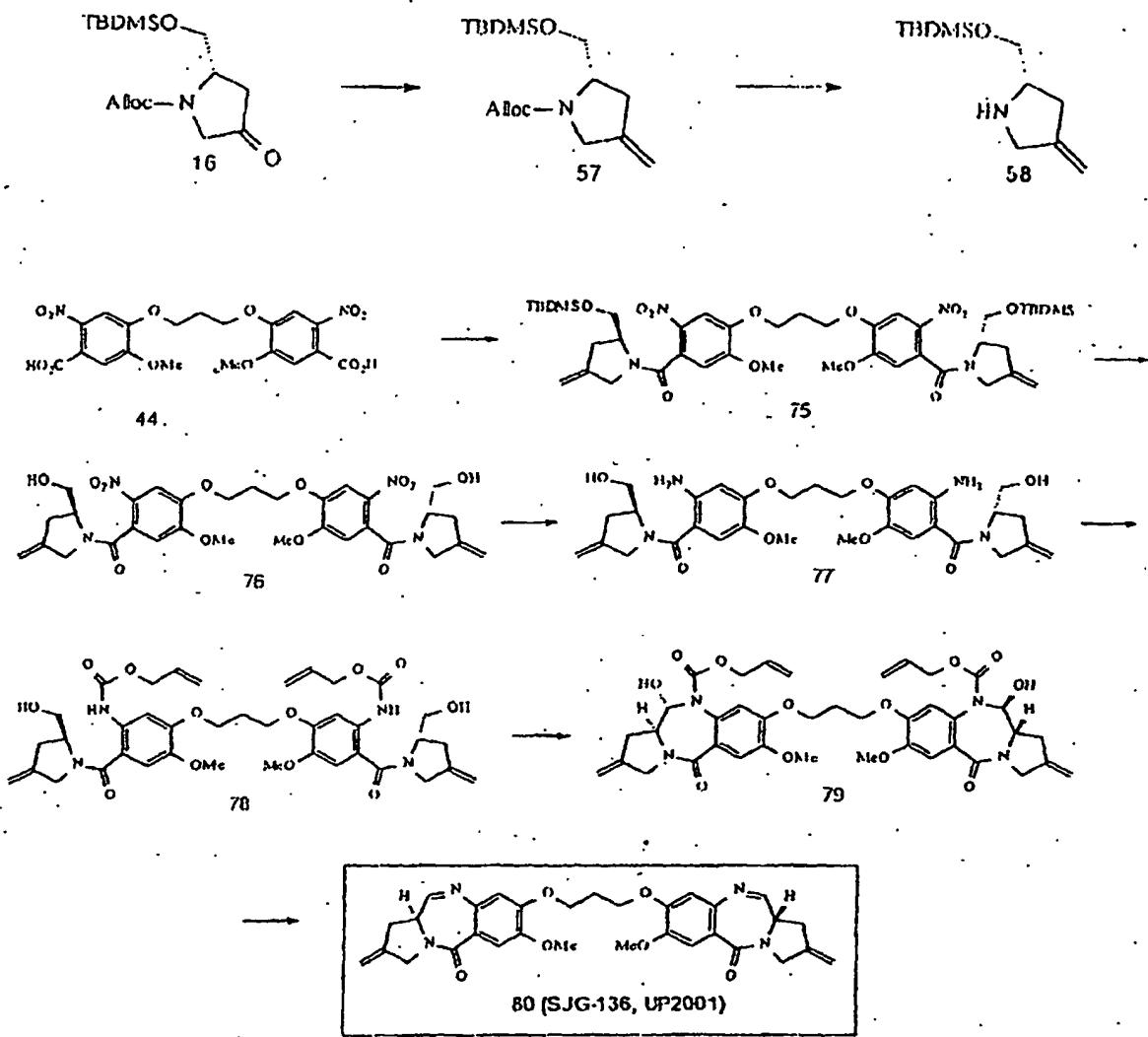
Figure 1

Figure 2a.

Figure 2b

Figure 3a

Figure 3b